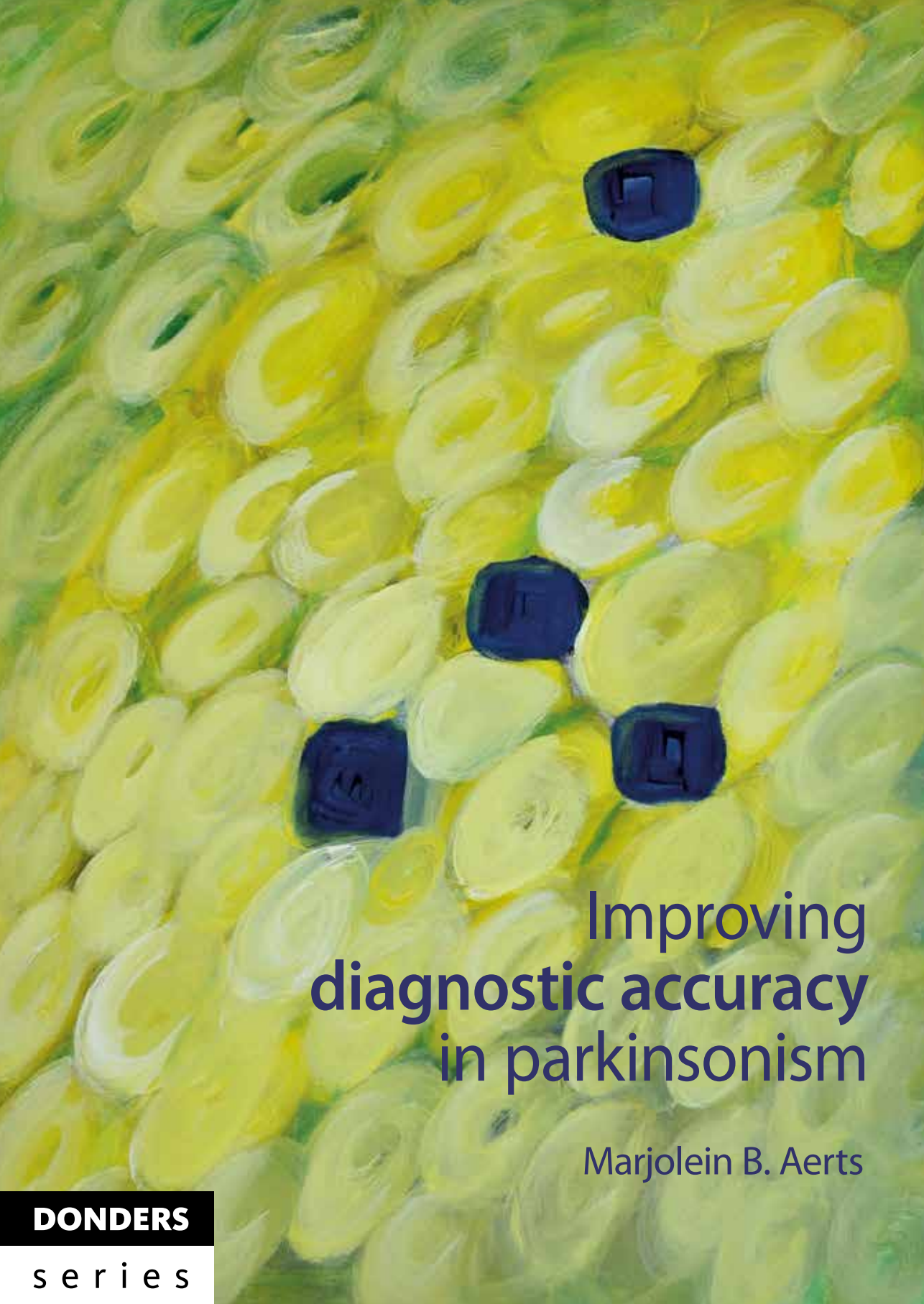

Improving **diagnostic accuracy** in parkinsonism

Marjolein B. Aerts

DONDERS
series

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Er zijn vaak duizend redenen om iets niet te doen;
juist die ene om het wel te doen moet goed genoeg zijn.

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Contents

1 Introduction and outline

Introduction

Parkinson's disease

Box 1 Clinical criteria of Parkinson's Disease (Hughes et al. 1992)

A. Diagnostic criteria

- Bradykinesia (slowness of initiation of voluntary movement with progressive reduction in speed and amplitude of repetitive actions
- And at least one of the following:
  - Muscular rigidity
  - 4-6 Hz rest tremor
  - Postural instability not caused by primary visual, vestibular, cerebellar, or proprioceptive dysfunction

B. Exclusion criteria for Parkinson's disease

- History of repeated strokes with stepwise progression of parkinson features
- History of repeated head injury
- History of definite encephalitis
- Oculogyric crises
- Neuroleptic treatment at onset of symptoms
- More than one affected relative
- Sustained remission
- Strictly unilateral features after 3 years
- Supranuclear gaze palsy
- Cerebellar signs
- Early severe autonomic involvement
- Early severe dementia with disturbances of memory, language, and praxis
- Babinski sign
- Presence of cerebral tumour or communicating hydrocephalus on CT scan
- Negative response to large dose of levodopa (malabsorption excluded)
- MPTP exposure

C. Supportive prospective positive criteria for Parkinson's disease

- Unilateral onset
- Rest tremor present
- Progressive disorder
- Persistent asymmetry affecting side of onset most
- Excellent response (70-100%) to levodopa
- Severe levodopa-induced chorea
- Levodopa response for 10 years or more
- Clinical course of 10 years or more

Parkinson's disease (PD) is the most common disorder in the group of parkinsonian syndromes, accounting for 75% of patients. The disease usually presents around the age of 60 years (but with a wide range), and is characterized by a slowly progressive, asymmetrical, dopamine-responsive hypokinetic-rigid syndrome. (Tolosa et al. 2006) However, although the diagnosis of PD is currently still based on the presence of such motor symptoms, these represent only the tip of the iceberg. (Langston 2006)

A broad variety of non-motor symptoms can accompany the characteristic motor symptoms, and this includes neuropsychiatric symptoms, sleep disorders and autonomic symptoms. With disease progression, these non-motor symptoms increase both in severity and frequency, (Hawkes 2008) although they are common even in de novo patients with PD. Some of these non-motor symptoms, like olfactory deficits, sleep problems and constipation, can even precede the characteristic motor symptoms by at least several years. (Chaudhuri and Naidu 2008)

Neuropathologically, PD is initially characterized by progressive loss of dopaminergic neurons in the substantia nigra caused by alpha-synuclein aggregations. Later in the course of the disease, the degeneration spreads in both brain stem and cerebrum. (Braak et al. 2003) Moreover, the pathology affects not only dopaminergic neurons, but also noradrenergic, cholinergic and serotonergic neurotransmitter systems.

The treatment of PD is only symptomatic, and no curative treatment is currently available. The most widely used approach is the administration of the dopamine precursor *levodopa*. In addition, drugs have been developed that stimulate dopamine receptors or that block the metabolism of endogenous or exogenous dopamine. Stereotactic deep brain surgery – and in particular deep brain stimulation with electrodes positioned in specific brain areas – can also improve clinical symptoms in selected patients. In addition to this medical management, attention is increasingly focused on a multidisciplinary team approach. (van der Marck et al. 2013a) No less than 19 professional disciplines can be involved, (Keus et al. 2012) and this involves (among others) speech-language therapy, physical therapy and occupational therapy. Parkinson nurses also play a vital role in the management of PD patients.

Evidence supporting the merits of allied health interventions is steadily growing, in particular for physical therapy (Keus et al. 2007; Keus et al. 2009) and speech-language therapy, (de Swart et al. 2003; Fox et al. 2012) but also for other disciplines. (Sturkenboom et al. 2013) Whether or not extra benefits are achieved by bundling the forces of several disciplines into a coherent multispecialty team approach remains unclear. One recent trial has shown that an integrated multidisciplinary approach offers benefits to patients, (van der Marck et al. 2013a) helping them to maintain a good quality of life as long as possible. However, another recent study – that tested a different model of integrated multidisciplinary care – showed only small benefits in favor of the intervention group, and these disappeared after correction for baseline differences. (van der Marck et al. 2013b) More work remains needed to identify the best team composition, and in particular to

identify which patients can reasonably be expected to benefit most from such an intensive and tailor-made team approach. (Parashos 2013)

Atypical parkinsonism

The group of atypical parkinsonism consists of multiple system atrophy, dementia with Lewy bodies, progressive supranuclear palsy and corticobasal degeneration. The first two disorders constitute, along with PD, the so-called *alpha synucleopathies*, because alpha synuclein aggregates are found upon neuropathological examination. Progressive supranuclear palsy and corticobasal degeneration are caused by tau aggregates and are therefore called *tauopathies*. In addition to these disorders, most neurologists would regard vascular parkinsonism as a form of atypical parkinsonism. I will briefly discuss the main features of these forms of atypical parkinsonism below, with references to key papers for further reading.

Multiple system atrophy

Multiple system atrophy (MSA) is characterized clinically by a combination of a symmetrical hypokinetic-rigid syndrome, cerebellar ataxia, autonomic disturbances and – less frequently – pyramidal tract involvement. Recent work has shown that patients can also develop cognitive impairment, in particular frontal executive dysfunction. (Brown et al. 2010) Patients show only a limited response to dopaminergic therapy, although initially a favorable response is possible. (Quinn 1989)

Clinically, MSA can be divided into two subtypes: MSA-c when the cerebellar symptoms are predominant, and MSA-p when the clinical picture is dominated by the hypokinetic-rigid symptoms. (Gilman et al. 2008) The disease typically presents around the age of 60, never before the age of 45, and is rapidly progressive. Within 5-6 years the majority of patients is wheelchair dependent. The mean disease duration is 8-10 years. (Klockgether et al. 1998; Watanabe et al. 2002; Wenning et al. 2013) Neuropathologically, the disease is characterized by alpha-synuclein inclusions in the striato-nigral (MSA-p) and/or olivoponto-cerebellar tracts (MSA-c). (Ozawa et al. 2004)

Box 2 Clinical criteria of Multiple system atrophy (Gilman et al. 2008)

A. Diagnostic criteria

- Possible diagnosis of MSA
  - Parkinsonism (bradykinesia with rigidity, tremor or postural instability) *or*
  - A cerebellar syndrome (gait ataxia with cerebellar dysarthria, limb ataxia or cerebellar oculomotor dysfunction) *and*
  - At least one feature suggesting autonomic dysfunction (otherwise unexplained urinary urgency, frequency or incomplete bladder emptying, erectile dysfunction in males or significant orthostatic blood pressure decline that does not meet the level required in probable MSA *and*
  - One additional feature (B)
- Probable diagnosis of MSA

  Autonomic failure involving urinary incontinence (inability to control the release of urine from the bladder, with erectile dysfunction in males) or an orthostatic decrease of blood pressure within 3 minutes of standing by at least 30mm Hg systolic or 15mmHg diastolic AND poorly levodopa-responsive parkinsonism (bradykinesia with rigidity, tremor or postural instability) OR a cerebellar syndrome (gait ataxia with cerebellar dysarthria, limb ataxia or cerebellar oculomotor dysfunction)
- Definite diagnosis of MSA

  Histopathological confirmation of the diagnosis is obtained at autopsy

B. Additional features of possible MSA

- Possible MSA-p or MSA-c
  - Babinski sign with hyperreflexia
  - Stridor
- Possible MSA-p
  - Rapidly progressive parkinsonism
  - Poor response to levodopa
  - Postural instability within 3 years of motor onset
  - Gait ataxia, cerebellar dysarthria, limb ataxia, or cerebellar oculomotor dysfunction
  - Dysphagia within 5 years of motor onset
  - Atrophy on MRI of putamen, middle cerebellar peduncle, pons or cerebellum
  - Hypometabolism on FGD-PET in putamen, brainstem or cerebellum
- Possible MSA-c
  - Parkinsonism (bradykinesia and rigidity)
  - Atrophy on MRI of putamen, middle cerebellar peduncle or pons
  - Hypometabolism on FDG-PET in putamen
  - Presynaptic nigrostriatal dopaminergic denervation on SPECT or PET

Box 2 Continued

C. Features supporting MSA

- Orofacial dystonia
- Disproportionate antecollis
- Camptocormia and/or Pisa syndrome
- Contractures of hands or feet
- Inspiratory sighs
- Severe dysphonia
- Severe dysathria
- New or increased snoring
- Cold hands and feet
- Pathologic laughter or crying

D. Features suggestive of alternative diagnoses

- Classic pill-rolling rest tremor
- Clinical significant neuropathy
- Hallucinations not induced by drugs
- Onset after age 75 years
- Family history of ataxia or parkinsonism
- Dementia (on DSM-IV)
- White matter lesions suggesting multiple sclerosis

Dementia with Lewy bodies

Box 3 Clinical criteria of Lewy body dementia (McKeith et al. 2005)

A. Diagnostic criteria

- Possible diagnosis of DLB
  Central feature with either one core feature or one suggestive feature
- Probable diagnosis of DLB
  Central feature with either two core features or one core feature and one suggestive feature
- Definite diagnosis of DLB
  Criteria for clinical DLB are met *and* histopathological confirmation of the diagnosis is obtained at autopsy

Box 3 Continued

B. Central feature

Dementia defined as progressive cognitive decline of sufficient magnitude to interfere with normal social or occupational function. Prominent or persistent memory impairment may not necessarily occur in the early stages but is usually evident with progression. Deficits of attention, executive function, and visuospatial ability may be specially prominent

C. Core features

- Fluctuating cognition with pronounced variations in attention and alertness
- Recurrent visual hallucinations that are typically well formed and detailed
- Spontaneous features of parkinsonism

D. Suggestive features

- REM sleep behaviour disorder
- Severe neuroleptic sensitivity
- Low dopamine transporter uptake in basal ganglia demostrated by PET or SPECT imaging

E. Supportive features

- Repeated falls and syncope
- Transient, unexplained loss of consciousness
- Severe autonomic dysfunction, e.g. orthostatic hypotension, urinary incontinence
- Hallucinations in other modalities
- Systemized delusions
- Depression
- Relative preservation of medial temporal lobe structures on CT/MRI scan
- Generalized low uptake on SPECT/PET perfusion scan with reduced occipital activity
- Abnormal (low uptake) MIBG myocardial scintigraphy
- Prominent slow wave activity on EEG with temporal lobe transient sharp waves

F. Non-supportive features

- The presence of cerebrovascular disease evident as focal neurologic signs or on brain imaging
- Other physical illness or brain disorder sufficient to account in part or total for the clinical picture
- If parkinsonism only appears for the first time at a stage of severe dementia

Dementia with Lewy bodies (DLB) is a form of dementia characterized by fluctuating cognition, visual hallucinations, a hypokinetic-rigid syndrome that is poorly responsive to dopaminergic medication, and autonomic disturbances. By definition, the cognitive disturbances precede the motor symptoms, or occur within 12 months of onset of the motor symptoms. (McKeith et al. 2005) An accompanying clinical feature is an increased

sensitivity to neuroleptics. The mean disease duration is 8-10 years. Neuropathologically, Lewy bodies can be found with alpha synuclein aggregations in the cortex, limbic system and brainstem. (McKeith et al. 2005)

Progressive supranuclear palsy

Box 4 Clinical criteria of Progressive supranuclear palsy (Litvan et al. 1996)

A. Diagnostic criteria

- Possible diagnosis of PSP

Gradually progressive disorder, onset at age 40 or later with *either* vertical (upward or downward) supranuclear palsy *or* both slowing of vertical saccades and prominent postural instability with falls in the first year of disease onset *and* no evidence of other diseases that could explain the foregoing features, as indicated by C. Mandatory exclusion criteria

- Probable diagnosis of PSP

Gradually progressive disorder, onset at age 40 or later with vertical (upward or downward) supranuclear palsy *and* prominent postural instability with falls in the first year of disease onset *and* no evidence of other diseases that could explain the foregoing features, as indicated by C. Mandatory exclusion criteria

- Definite diagnosis of PSP

Clinically probable or possible PSP and histopathological evidence of typical PSP

B. Features supporting PSP

- Symmetric akinesia or rigidity, proximal more than distal
- Abnormal neck posture, especially retrocollis
- Poor or absent response of parkinsonism to levodopa therapy
- Early dysphagia and dysarthria
- Early onset of cognitive impairment including at least two of the following: apathy, impairment in abstract thought, decreased verbal fluency, utilization or imitation behavior, or frontal release signs

C. Mandatory exclusion criteria

- Recent history of encephalitis
- Alien limb syndrome, cortical sensory deficits, frontal focal or temporoparietal atrophy
- Hallucinations or delusions unrelated to dopaminergic therapy
- Cortical dementia of Alzheimer's type (severe amnesia and aphasia or agnosia according to NINCDS-ADRA criteria)
- Prominent, early cerebellar symptoms or prominent, early unexplained dysautonomia (marked hypotension and urinary disturbances)
- Severe asymmetric parkinsonian signs
- Neuroradiologic evidence of relevant structural abnormality
- Whipple's disease

Progressive supranuclear palsy (PSP) is classically characterized clinically by a symmetrical hypokinetic-rigid syndrome with postural instability, causing frequent falls early in the cause of the disease, cognitive deterioration (mainly frontal executive dysfunction) and a supranuclear gaze palsy (Steele-Richardson-Olszewski syndrome). However, an asymmetric variant, closely resembling PD also exists (PSP-P). The mean age at which symptoms appear is between 50 and 70 years. The disease is rapidly progressive, with a mean survival of 6-9 years after diagnosis. (Burn and Lees 2002) The response to symptomatic treatment is rarely gratifying. Neuropathologically the disease is characterized by hyper-fosforylated tau protein depositons with loss of neurons in the midbrain and frontal lobes. (Dickson et al. 2007)

Box 5 Clinical criteria of Corticobasal syndrome (Boeve et al. 2003)

A. Core features

- Insidious onset and progressive course
- No identifiable cause (e.g. tumor, infarct)
- Cortical dysfunction as reflected by at least one of the following: focal or asymmetrical ideomotor apraxia, alien limb phenomenon, cortical sensory loss, visual or sensory hemineglect, constructional apraxia, focal or asymmetrical myoclonus, apraxia of speech/nonfluent aphasia
- Extrapyramidal dysfunction as reflected by at least one of the following: focal or asymmetrical appendicular rigidity lacking prominent and sustained levodopa response OR focal or asymmetrical appendicular dystonia

B. Supportive investigations

- Variable degrees of focal or lateralized cognitive dysfunction, with relative preservation of learning and memory, on neuropsychometric testing
- Focal or asymmetric atrophy on CT or MRI, typically maximal in parietofrontal cortex
- Focal or asymmetric hypoperfusion on SPECT and PET, typically maximal in parietofrontal cortex and/or basal ganglia and/or thalamus

Corticobasal degeneration

Corticobasal degeneration is characterized clinically by an asymmetrical, non-dopamine responsive hypokinetic-rigid syndrome, with additional features including dystonia, and asymmetrical cortical dysfunction (apraxia, aphasia, stimulus-sensitive myoclonus, cortical sensory disturbances or an alien limb phenomenon). (Mahapatra et al. 2004) This symptom complex is known as corticobasal syndrome (CBS), which can be caused not only by CBS but also by a number of other conditions (e.g. vascular lesions or prion disease). Clinical differentiation can be complex, as both MSA (Batla et al. 2013) and PSP can present quite asymmetrically, and CBS on the other hand can present symmetrically. (Wadia and

Lang 2007) The response to symptomatic treatment is again poor. Symptoms usually present around the age of 60 and the disease is rapidly progressive, with a mean survival of 8 years after disease onset. (Wenning et al. 1998) Neuropathologically, the disease is characterized by asymmetrical atrophy of both frontal and parietal cortex with aggregations of hyper-fosforylated tau protein. (Ludolph et al. 2009)

Vascular parkinsonism

Vascular parkinsonism (VaP), is characterized by the combination of a hypokinetic rigid syndrome and evidence of cerebrovascular disorder. Lower body parkinsonism is the predominant feature in up to two thirds of the patients. Patients are older upon presentation than patients with PD (mean age of 70), and the mean survival is 10-12 years. Vascular risk factors are present in almost 90% of the patients with VaP. The course of the disease can be abrupt, or stepwise, but insidious courses are not uncommon (70%). Sense of smell is typically preserved. Over one half of the patients show at least some response to levodopa therapy. (Glass et al. 2012)

Other look-alikes

There are many other diseases that can present with parkinsonism, or develop parkinsonism in the course of the disease. One example is the development of parkinsonism in the course of Creutzfeldt Jakob's disease. On the other hand, there are many conditions that may mimic (aspects) of a hypokinetic-rigid syndrome, such as the lack of facial expression in a patient with depression. In chapter 2 we will discuss this in more detail.

Box 6 Clinical criteria for Vascular Parkinsonism (Zijlmans et al. 2004)

A. Parkinsonism: bradykinesia and at least one of the following: rest tremor, muscular rigidity, or postural instability (not caused by primary visual, vestibular, cerebellar or proprioceptive dysfunction.

B. Cerebrovascular disease, defined by evidence of relevant cerebrovascular disease by brain imaging (CT or MRI), *or* the presence of focal signs or symptoms that are consistent with stroke.

C. A relationship between the above two disorders. In practice: An acute or delayed progressive onset with infarcts in or near areas that can increase the basal ganglia motor output (GPe or substantia nigra pars compacta) or decrease the thalamocortical drive directly (VL of the thalamus, large frontal lobe infarct). The parkinsonism at onset consists of a contralateral bradykinetic rigid syndrome or shuffling gait, within 1 year after a stroke. Or, an insidious onset of parkinsonism with extensive subcortical white matter lesions, bilateral symptoms at onset, and the presence of early shuffling gait or early cognitive dysfunction.

Box 6 Continued

D. Exclusion criteria for VaP: History of repeated head injury, definite encephalitis, neuroleptic treatment at onset of symptoms, presence of cerebral tumor or communicating hydrocephalus on CT or MRI scan, or other alternative explanation for parkinsonism.

Differentiating between PD and atypical parkinsonism

Clinical criteria (boxes 1-6) are available for the diagnosis of PD and for the various forms of atypical parkinsonism. These criteria provide a 'possible' and 'probable' diagnosis. (Litvan et al. 1996) This distinction is based globally on the following items: rate of progression (slow for PD, rapid for AP); response to dopaminergic treatment (good and sustained for PD; absent or at best modest but short-lived for AP; and presence of red flags (absent for PD; one or more for AP, with sometimes characteristic combinations). The term 'red flags' refers to all symptoms that signal the presence of AP (and at times the presence of a specific form of AP), such as ataxia or vertical gaze palsy. The gold standard, however, remains a neuropathological confirmation. Clinicopathological studies have shown that, compared to this golden standard, a relatively large proportion of clinical diagnoses is incorrect. Up to 10% of patients diagnosed with PD during life has a different neuropathological diagnosis upon post-mortem examination. (Hughes et al. 2001a; Hughes et al. 2001b; Hughes et al. 2002) In atypical parkinsonism, this percentage is even higher. Only 70 % of the neuropathologically proven cases of MSA and/or PSP were correctly diagnosed as such during life. (Litvan et al. 1997; Osaki et al. 2002; Williams et al. 2005) Due to increasing awareness of these syndromes, the number of correct diagnoses is increasing. The clinico-pathological study performed in 2002 by Hughes et al (Hughes et al. 2002) illustrates this: the positive predictive value for the diagnosis of PD was 98.6% (sensitivity 91.1% specificity 98.4%). The diagnostic accuracy of the atypical parkinsonian syndromes has also increased; the use of the clinical consensus criteria leads to a sensitivity of 88.2% and a specificity of 95.4%. For PSP a sensitivity of 84.2% and a specificity of 96.8% were found and for CBS a sensitivity of 25% and a specificity of 98,6%. (Hughes et al. 2002) Moreover, these numbers have been validated for neurologists specialized in movement disorders and after extensive follow up (3 years). The diagnostic dilemma in differentiating between PD and AP is much greater in the first year of the disease and in general hospitals: the sensitivity for the diagnosis of MSA upon the first visit was only 25%, and only 50-60% after 1 year of follow-up. (Litvan et al. 1997; Osaki et al. 2002)

Aim and outline of the thesis

The overall aim of this thesis is to evaluate and optimize the differentiation between PD and atypical parkinsonism in a clinical setting.
First, the clinical evaluation of a patient with parkinsonism is described in detail in **chapter 2**, including careful assessment of potential 'red flags'. In **chapter 3** we have explored the potential of CSF biomarkers in the differentiation between PD and different forms of atypical parkinsonism, as well as between different forms of atypical parkinsonism. In the last part of this thesis, in **chapter 4**, we present the results of a prospective clinical study in which we evaluated both clinical and ancillary investigations differentiating PD from AP. We will conclude with several recommendations on how to incorporate these findings in current clinical practice.

2 Clinical evaluation of a patient with parkinsonism

Based on

Aerts MB, Esselink RA, Post B, van de Warrenburg BP, Bloem BR. Improving the diagnostic accuracy in parkinsonism: a three-pronged approach. Practical Neurology. 2012;12(2):77-87.

Summary

Separating Parkinson's disease (PD) from the various causes of atypical parkinsonism (AP) is a common and clinically relevant challenge in clinical practice. Distinguishing between the different causes of AP is even more difficult. Here we discuss a systematic, clinically based and three-pronged approach that can assist clinicians in establishing the correct diagnosis in the consulting room. The three consecutive steps include: 1. to verify that the clinical syndrome truly represents parkinsonism (hypokinetic-rigid syndrome); 2. to systematically search for the presence of 'red flags' (alarm signs that may signal the presence of AP); and 3. to integrate these two steps, as a basis for a narrow differential diagnosis and guide for further ancillary tests.

Introduction

The presence of parkinsonism can be established in the clinical examination room, based on history taking and neurological examination. Parkinson's disease (PD) is the most frequent cause, but the differential diagnosis is broad and includes, among others, multiple system atrophy (MSA), progressive supranuclear palsy (PSP), corticobasal syndrome (CBS), dementia with Lewy bodies (DLB), vascular parkinsonism (VaP) and drug-induced parkinsonism. This wide group of 'other' causes of parkinsonism is here referred as atypical parkinsonism (AP). A list of the potential causes of parkinsonism is presented in Table 1a and b.

Table 1a Neurodegenerative diseases known to possibly present with features of parkinsonism

Parkinson's Disease (idiopathic)
Genetic parkinsonism, e.g. LRRK2, parkin, PINK1
Atypical Parkinsonism
- Multiple system atrophy (MSA)
- Progressive supranuclear palsy (PSP)
- Corticobasal syndrome (CBS)
- Lewy body dementia (DLB)
Alzheimer's Disease with parkinsonism
Frontotemporal dementia with parkinsonism (FTDP-17)
Huntington's Disease
Spinocerebellar ataxias (SCA), e.g. SCA2, 3 and 17
Recessive parkinsonism-dystonia disorders (e.g. Kufor-Rakeb, PARK14, DYT16)
Rapid-onset dystonia-parkinsonism
Dopa-responsive dystonia
Neuroacanthocytosis
Fragile-X tremor/ataxia syndrome
X-linked dystonia-parkinsonism (Lubag)
Fahr syndrome
Neuronal intranuclear inclusion disease
Neurofilament inclusion body disease
Perry syndrome (Dynactin mutations)
Hereditary spastic paraplegia (SPG11)
Heredodegenerative diseases
- Wilson's disease
- Gaucher's disease type I
- Mitochondrial disorders
- NBIA (neurodegeneration with brain iron accumulation)

Table 1b Secondary causes of parkinsonism

Medication induced	
- Neuroleptics	- Lithium
- Valproic acid	- Calcium channels blockers
Structural	
- Vascular (vascular parkinsonism)	- Subdural hematoma
- Trauma (dementia pugilistica)	- Normal pressure hydrocephalus
Toxins	
- Manganese	- Carbon monoxide
- MPTP	- Carbon disulfide
- Mercury	- Cyanide
- Methanol	- Organophosphates
- Solvents	- Pesticides
(Post) infectious	
- HIV	- Measles
- Encephalitis lethargica	- EBV (Epstein Barr virus)
- Japanese B encephalitis	- West-nile virus
- Coxsackie B virus	- Neurosyphilis
Metabolic	
- Hypoparathyroidism	- GM1 gangliosidosis
- Hypothyroidism	- Addison's disease
- Uremia	- Hypoxia
- Carbon monoxide	

Parkinsonism is a common finding in neurological outpatient clinics. The incidence ranges from an estimated 0.5/1000 person-years for patients aged between 55 and 65 years to 10.6/1000 person-years for subjects aged above 85 years. (de Lau et al. 2004) Early in the course of the disease, establishing a correct diagnosis can be challenging due to overlap in the clinical presentation between the various forms of parkinsonism (Figure 1). However, being able to differentiate between PD and AP is highly relevant, for several reasons. First, patients find it important to know which specific disease they have. Second, the prognosis varies greatly across the different causes of parkinsonism. In general, disease progression is slower in PD compared with AP. Third, an accurate diagnosis is important to prompt attention to disease-specific complications, such as nocturnal inspiratory stridor in MSA patients (which is a cause of sudden death in this disorder!), or the 'motor recklessness' and concomitant injurious falls seen in patients with PSP. Medication effects are typically much less in AP, with fewer patients that respond, and a more modest and temporary improvement for those that respond. Fourth, differentiation between PD and AP is

important for research purposes, as a correct diagnosis is needed to include the right patients for specific trials.
A recent paper in this journal carefully discussed the essentials of PD. (Lees 2010) Capitalizing on this paper, we here present a framework to differentiate between PD and AP, based on three subsequent steps.

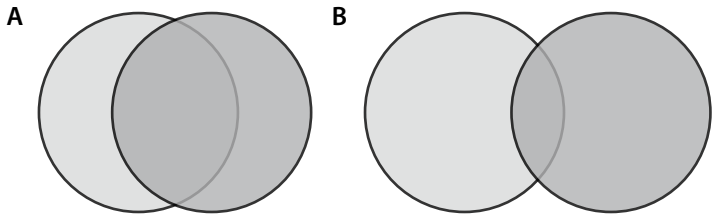

Figure 1 Overlapping circles depict the overlap in clinical symptoms. (**A**) Early in the course of the disease the symptoms of Parkinson's disease (light grey) and atypical parkinsonism (dark grey) can closely resemble each other (10-20% overlap), whereas (**B**) late in the course of the disease a small percentage (5%) is still misclassified, even in the hands of experts.

Step 1: the core criteria of parkinsonism

The first step is to carefully assess whether the patient truly has parkinsonism. This can be overlooked easily, for two reasons. First, the motor symptoms of parkinsonism can be subtle early in the course of the disease, leading to a false-negative diagnosis. Second, some conditions mimic parkinsonism, leading to a false-positive diagnosis (Table 2). We will exemplify both types of misdiagnosis.
The conventional core criteria for parkinsonism (UK Brain Bank) include bradykinesia and at least one of the following: rigidity, rest tremor or postural instability. Hence, the presence of bradykinesia is a prerequisite for the diagnosis of parkinsonism. Classic bradykinesia is defined as a progressive decrement of both the speed and amplitude of repetitive movements. Clumsiness, slowness or irregularity of movements is, according to this definition, insufficient to fulfill the criterion 'bradykinesia'. Indeed, patients with e.g. cerebellar ataxia generally exhibit irregular movements, while patients with spasticity can have slow movements, but both lack the classical decrement that is characteristic for true bradykinesia. This may explain why patients with an upper motor neuron presentation of amyotrophic lateral sclerosis are sometimes misdiagnosed as having parkinsonism. Similarly, the spasticity observed in patients with an upper motor neuron disease can be mistaken for the rigidity that is observed in parkinsonism. The clasp-knife phenomenon

(resistance building up upon passive stretch, which is suddenly giving way) and the speed-dependency of the increased tone (present in spasticity and absent in rigidity) can be used to discriminate between true rigidity and increased tone based due to spasticity.

The presence of asymmetrical resting tremor is widely perceived as a classical presentation of PD, but not all patients actually have parkinsonism. Studies that used nuclear imaging to visualize the dopaminergic pathway have identified subjects with such asymmetrical resting tremor but without evidence of a dopaminergic deficit upon imaging, so called SWEDD's (scans without evidence of dopamine deficit). The underlying etiology of tremor in these SWEDD's is often dystonia. Separating (rest) tremor in patients with SWEDD's from parkinsonian rest tremor can be very challenging. Indeed, some SWEDD's have inadvertently been included in clinical drug studies designed for patients with parkinsonism. Table 3 summarizes certain clinical features that help to discriminate between tremor in PD and SWEDD's. (Bajaj et al. 2010)

Table 2 Conditions mimicking parkinsonism

Signs and symptoms	Differential diagnosis
Slow execution of movements	- Decreased motor control (e.g. spasticity or ataxia) - Cognitive causes (e.g. apathy) - Mood (depression) - Metabolic causes (e.g. hypothyroidism) - Psychogenic slowness
Lack of spontaneous movements	- Diminished arm swing (e.g. dystonia or frozen shoulder syndrome) - Mask-like face in depression - Psychogenic causes
Cog wheel phenomenon	- Essential tremor - Drug-induced tremor
Classical resting tremor	- Dystonic tremor

Table 3 Differentiating SWEDD's and PD

Symptom	PD or SWEDD's
Bradykinesia (i.e. both slowness AND reduction of amplitude)	PD
Tremor characteristics	
- Resetting phenomenon (re-emergent tremor)	PD
- Thumb extension tremor	
- Head tremor	SWEDD's
- Predominantly present in rest	PD
- Predominantly positional	PD
- Task-/position-specificity	SWEDD's
- Jerkiness	SWEDD's
	SWEDD's
Adequate response to levodopa treatment	PD >> SWEDD's
Non-motor symptoms, including olfaction disturbances	PD

PD: Parkinson's disease; SWEDD's: scans without evidence of dopaminergic deficits

Step 2: presence of 'red flags'

Once the presence of parkinsonism has been established, the second step is to thoroughly search for the presence of additional symptoms and signs. These additional symptoms – which serve to signal the presence of AP – are commonly referred to as the 'red flags' for AP. Each of these additional symptoms will be described in more detail below. In addition, we have listed the main red flags in Table 4, including suggestions for diagnoses that can be considered when a specific red flag is present.

Motor symptoms

Classically, the distribution of symptoms in PD is asymmetrical. Although these symptoms will inevitably present bilaterally, this asymmetrical nature persists throughout the course of the disease. In contrast, symptoms are generally much more symmetrical in patients with AP. The separation is never complete, as some patients with e.g. MSA can present with an asymmetric distribution. And there is one important exception to the rule: the asymmetry in CBS is even more pronounced than in PD. A lower-body distribution of symptoms – where the legs are much more affected than the arms – is the textbook presentation of VaP, especially when accompanied by a stepwise pattern of disease progression. A very pronounced axial distribution, in which the axis (i.e. neck, back) is much more affected than the extremities, would also be atypical for PD and more

suggestive of PSP. Disease progression is generally slow in PD, whereas AP can progress more rapidly. Consequently, life-time expectancy in AP is often reduced compared to PD. We will next discuss several specific motor symptoms in more detail.

Tremor characteristics

PD classically presents with an asymmetric rest-tremor, and it is important to document whether a true pill-rolling type (with involvement of the thumb) is present, as this is very supportive of a diagnosis of PD. Pill-rolling tremor does not exclude AP completely, because it can also be observed in drug-induced parkinsonism and MSA, although the tremor tends to present more bilaterally and symmetrically in these two conditions. So tremor symmetry is a valuable distinguishing feature, next to the nature of the tremor. Again, the separation is not complete, because an asymmetric, pill-rolling rest tremor can occur in patients with DLB and sporadically even in so called PSP-P patients. A jerky tremor, possibly due to polyminimyoclonus, is more suggestive of MSA.

Severe essential tremor, Holmes tremor and dystonic tremor can present with a profound rest component. Several elements may help in separating these tremor types from parkinsonian tremor. Essential tremor is very symmetrical, and no gross other neurological abnormalities should be present (except for mild gait ataxia). Table 3 lists several characteristics of dystonic tremor. In addition, dystonic tremor more typically presents with thumb extension from the neutral position, while parkinsonian rest tremor usually involves thumb flexion from neutral. Holmes tremor tends to have a lower frequency (at around 3 Hz) than tremor is PD (generally 4-6 Hz), but reliable frequency-based discrimination between the various tremor etiologies is difficult due to considerable overlap in frequencies. (Abdo et al. 2010)

When observing rest tremor, it is important to specifically search for the resetting phenomenon (also referred to as re-emergent tremor): the re-emergence of tremor with a latency after a positional change. This latency suggests that a rest tremor has now reappeared in the newly acquired position, and this should not be mistaken for a positional tremor. Absence of this resetting phenomenon might point towards a dystonic tremor (Table 3). (Bajaj et al. 2010)

Myoclonus

This concerns sudden, brief, shock-like movements, caused by muscle contractions. Especially when myoclonus is severe and frequently repetitive or even rhythmic, the differentiation with tremor can be challenging. A useful sign is the abruptness of the movements, because myoclonus is typically jerky, and tremor is not. Careful examination of the outstretched hands can reveal subtle polyminimyoclonus without a resetting phenomenon and with stimulus-sensitivity (touch of the outstretched fingers); this is suggestive of MSA. More pronounced myoclonus, most prominent upon voluntary action or in response to sensory stimuli, can be observed in DLB, CBS, and rarely PSP. In CBS, the

observed myoclonus is generally focal and often confined to one limb. Myoclonus can also be observed in spinocerebellar ataxia syndromes, particularly SCA2, as well as in genetic parkinsonian syndromes like PARK9. (Abdo et al. 2010)

Dysphagia / dysarthria

Mild dysphagia and dysarthria can manifest in all parkinsonian disorders. However, the presence of severe dysphagia early in the course of the disease is suggestive for PSP, CBS and MSA, especially if a percutaneous endoscopic gastrostomy is necessary. Similarly, early presence of severe dysarthria should prompt to MSA or PSP as possible diagnosis. The specific characters of the dysarthria may help to differentiate these two disorders: a pseudobulbar component suggests PSP, while cerebellar dysarthria suggests MSA. Interrater agreement for these dysarthric features is admittedly poor, perhaps with the exception of a high-pitched, quivery voice, which is fairly characteristic and suggestive for MSA. (Gilman et al. 2008)

Dystonia

This can occur in both PD and AP. Focal dystonia can be seen in patients with PD, especially in patients with young-onset PD or one of the recessive forms of parkinsonism. This mainly involves dystonia of the foot, and can be dopamine responsive (hence the frequent occurrence early in the morning during a deep off phase). Different forms of dystonia are seen in patients with AP. Focal dystonia of the neck muscles (retrocollis) or the eyelid muscles (blepharospasm) occurs in PSP. Focal dystonia of the neck muscles also occurs in MSA, but now in the form of a mixed antecollis-laterocollis (often fixed and painful). MSA can also present with focal dystonia of the vocal cords (spasmodic dysphonia), or a segmental dystonia of trunk muscles, causing lateroflexion (Pisa sign) or anteroflexion (camptocormia) of the trunk. (Doherty et al. 2011) Note that a Pisa sign or camptocormia can also occur in PD or as a side effect of drugs (mainly neuroleptics). A full differential diagnosis is presented in table 4. The presence of early, fixed limb dystonia suggests a diagnosis CBS. (Boeve et al. 2003)

Pyramidal involvement

Pathological reflexes, including hyperreflexia and Babinski's sign, can be seen in VP and MSA. The Babinski sign should be distinguished from the striatal toe, which reflects dystonia of the extensor halluces longus muscle (common in PD). Differentiation can be difficult, but generally a sustained extensor response is indicative for a striatal toe, while a Babinski response includes an immediate return of the big toe to the neutral position after striking the foot sole. The Babinski response is also associated with fanning of the other toes, unlike the striatal toe. Furthermore, a striatal toe can present spontaneously, especially during walking on bare feet.

Table 4 Red flags and their most likely diagnosis

Signs and Symptoms	Most likely diagnosis
Pattern of distribution	
- Symmetrical	PSP, MSA
- Asymmetrical*	CBS (very asymmetrical)
- Lower-body phenotype	VaP
Course of the disease	
- Rapid progression (H&Y 3 < 5 years)	PSP, MSA
- Stepwise progression	VaP
- Remission	VaP, drug-induced parkinsonism
Medication	
- No/insufficient response to levodopa (>1g per day of levodopa over 1 month)	No response: PSP, CBS; partial response: MSA
- Early/profound levodopa intolerance	DLB, VaP
- Levodopa-induced dyskinesia*	MSA, DLB, VaP
- Non-dopa responsive pain	All forms of AP
Tremor	
- Asymmetrical pill-rolling tremor*	seldom: MSA
- Irregular, jerky tremor	MSA, CBS
Myoclonus	MSA (outstretched fingers), CBS, PSP, DLB, SCA 2, PARK9
Dysphagia and dysarthria	
- Early, severe dysarthria	AP
- Early, severe dysphagia	PSP, MSA
- Dysphonia (spasmodic)	MSA
Sensory disturbances	
- Cortical	CBS - Drug induced: amantadine - Intoxication : carbon-disulfide, manganese, solvents, carbon-monoxide - Infectious: syphilis, HIV - Paraneoplastic: parkinsonism and polyneuropathy (fast progression!) - Endocrine: hypoparathyreoidism - Metabolic: gangliosidosis - Mitochondrial: MERFF, POLG mutation - Neurodegenerative: Neuronal intranuclear inclusion disease
- Polyneuropathy	MSA

Table 4 Continued

Signs and Symptoms	Most likely diagnosis
Psychiatric symptoms	
- Apathy (early)*	PSP
- Disinhibition	
· Emotionally	Early: PSP, to lesser extent: MSA
· Pseudo-bulbar disinhibition	PSP, CBS
Hallucinations, delusions	DLB (early)
Pyramidal involvement	VaP, MSA, PARK2,9
Ataxia (cerebellar)	MSA, SCA 2,3,17, Neuronal intranuclear inclusion disease
Eye movement disturbances	
- Supranuclear palsy	PSP
- Round-the-house-phenomenon	PSP
- Saccadic eye movements	
· Delayed initiation	CBS
· Delayed execution	PSP
- Gaze impersistance	MSA, SCA, PSP
- Square wave jerks	MSA, SCA, PSP
- Dysmetria/overshoot	MSA, SCA
- Nystagmus	MSA, SCA
- Ocular apraxia	CBS
Oculogyric crisis	Drug-induced parkinsonism (anti-psychotics, anti-emetics), juvenile parkinsonism, bilateral thalamic lesions
Dystonia	
- Orofacial	MSA, PSP (blepharospasm), drug-induced
- Cervical	MSA (antecollis), PSP (retrocollis)
- Axial	
· Pisa sign*	- MSA - Drug induced (both typical and atypical anti-psychotics, anti-depressants, anti-emetics, choline-esterase inhibitors, dopaminergic medication
· Camptocormia*	- Spine deformities; scoliosis · MSA · Alzheimer's disease · Myopathy, myasthenia, CIDP) · Drug-induced · Spine deformities, arthritis · Paraneoplastic

Table 4 Continued

Signs and Symptoms	Most likely diagnosis
- Limbs* - Generalized	MSA, drug-induced - MSA - CBS - Hereditary parkinsonism (PARK 1,2,6,7,9,14 - Hereditary dystonia syndromes (DYT 3,5,12,16, SCA3 - Intoxications: neuroleptics, carbon-monoxide, manganese - Accumulation diseases: Wilson's disease NBIA1 - Miscellaneous: hemi-parkinsonism-hemi-dystonia, neuroacanthocytosis, Huntington's disease
- Fixed	CBS (early), MSA (late in the course of the disease)
Gait and balance disorders - Early postural instability Use of walking aids/ wheelchair dependency*	 PSP; to a lesser extent: MSA, CBS and VaP < 3 yrs: MSA, PSP 3-10 yrs: other forms of AP
Sleep disturbances - REM sleep behavior disorder - Sleep apnea syndrome Nocturnal inspiratory stridor	 PD, MSA, DLB MSA MSA
Cognitive dysfunction - Early and profound - Relatively late* - Relatively mild cognitive dysfunction - Apraxia - Aphasia	 PSP, DLB, FTD, Huntington's Disease, NPH CBS, VaP MSA CBS, PSP (to a lesser extent) CBS, PSP (to a lesser extent)
Autonomic dysfunction - present early and severely - cold, discolored extremities ('cold hands sign')	 MSA, DLB (to a lesser extent) MSA

MSA, multi system atrophy; PSP, progressive supranuclear palsy; DLB, diffuse Lewy body disease; CBS, corticobasal syndrome; VaP, vascular parkinsonism; FTD, frontotemporal dementia; NPH, normal pressure hydrocephalus; REM, rapid eye movement; SCA, spinocerebellar ataxia.
*might also be present in Parkinson's Disease

Ataxia

This can result from cerebellar or sensory dysfunction, and an ataxic gait can also be observed in patients with frontal or vestibular pathology. The presence of ataxia, especially cerebellar ataxia markedly affects the differential diagnosis, and even mild ataxia calls for further investigation. (Gilman et al. 2008) The differential diagnosis of parkinsonism plus ataxia includes MSA, SCA (2, 3 and 17) and Fragile-X –tremor-ataxia-syndrome (FXTAS). The majority of patients with MSA present with predominant parkinsonism (MSA-p), but up to 20 percent of cases can present with mainly a cerebellar syndrome (MSA-c). (Gilman et al. 2008) In Japan, MSA-c is the more common form. Most, but not all MSA patients will ultimately exhibit both parkinsonian and cerebellar features, although marked bradykinesia and hypokinesia can make it difficult to detect ataxia in severely affected patients. Cerebellar features can also be observed in patients with PSP, typically as square-wave jerks, but also as true cerebellar ataxia in up to 15% of patients. Genetic and ethnic variability affect this ataxic presentation in PSP, as it is most prevalent in Japan. (Kanazawa et al. 2009)

Gait and balance disorders

Disturbed gait can be observed in generally all patients with parkinsonism, especially late in the disease course, but the pattern varies between different diseases. Early gait abnormalities in PD include slowing of gait and an asymmetrically reduced arm swing. With disease progression, step height and length become smaller, and many patients develop start hesitation, festination (defined as taking increasingly rapid and small sequential steps during walking) and freezing (defined as debilitating episodes during which they are unable to start walking or, while walking, suddenly fail to continue moving forward). (Snijders et al. 2008) Importantly, gait remains narrow-based in PD, even until late in the disease, but is usually broad-based for patients with the various forms of AP, even early on in the disease. Freezing tends to occur much earlier and more severely in patients with AP, and unlike PD responds less well (or not at all) to external cueing. The exception is drug-induced parkinsonism, where freezing seems very rare. Ataxic gait is a key symptom in MSA-c. Gait in CBS is typically affected by asymmetrical and usually fixed foot dystonia. Balance is generally not disturbed in early PD, unlike the situation in most forms of AP. Early falls (within the first year of the disease) are a hallmark of PSP, and are caused by a combination of gait and balance problems. (Litvan 2001) Most falls are backward (for reasons unknown), whereas PD patients mostly fall forward (due to freezing of gait). Unlike PD, falls in PSP often cause serious injuries – fractures of longbones and head injury – because many patients are also reckless, and because protective responses during falls are absent. Although characteristic, falls within the first year of disease are not exclusive for PSP, can also occur in MSA, CBS, DLB and other forms of parkinsonism. Typical for MSA is the presence of vertical ('drop down') falls due to preceding syncope (orthostatic hypotension), while this is rare in PD. Patients with AP often require walking aids, and may

become fully wheelchair-bound within the first three years of the disease. This 'wheelchair sign' would be very unusual for PD. (Gilman et al. 2008)
Gait and balance are best assessed using a combination of tests, including a proper gait analysis and a retropulsion test (incorporated in UPDRS III). (Jacobs et al. 2006) Another good test is tandem gait (performed by instructing patients to walk 10 consecutive steps along a thin line); the test is usually normal in PD, and taking even a single corrective side step points towards AP. (Abdo et al. 2006) A related test is asking whether patients are still able to ride their bicycle: cycling is usually preserved in early PD, but inability to cycle early in the disease course suggest AP. (Aerts et al. 2011a)

Eye movement disorders

Examination of the eye movements is crucial for the clinical diagnostic process, as PD and the different forms of AP can show different abnormalities (although these can be subtle). A thorough examination of eye movements involves observing fixation, voluntary eye movements in all directions, smooth pursuit, and saccades in both the horizontal and vertical direction. If a vertical gaze palsy is observed, it is important to investigate the doll-eye-phenomenon to localize the disorder as being either supra- or (infra)nuclear. An upward vertical gaze palsy can be observed in healthy elderly, albeit with preserved velocity of the residual saccade. Also, in PSP downgaze is usually impaired before upgaze. Supranuclear vertical gaze palsy is characteristic for PSP when present, but a considerable proportion of patients never develop this. (Litvan 2001) Overt supranuclear vertical gaze palsy can be preceded by slowing of vertical saccades and, even more subtle, the 'round-the-house' phenomenon (abnormal trajectory with subtle deviation from the straight line upon attempted vertical saccades, presumably the first stage of saccadic slowing). Cerebellar eye movements, including square wave jerks, can also be observed in PSP patients. (Kanazawa et al. 2009)
MSA patients can show cerebellar eye movements, with nystagmus, square-wave jerks and dysmetria, but often also hypometric saccades as a reflection of concurrent parkinsonism. In CBS the initiation – but not the execution – of saccades can be slowed in both the horizontal and vertical plane, whereas PD patients show limited spontaneous blinking and hypometric eye movements, but a preserved velocity of the saccades. (Kennard 2007)

Autonomic dysfunction

Symptoms of autonomic dysfunction, mostly in the form of orthostatic hypotension, erectile dysfunction, and urge incontinence are common, early symptoms of MSA, usually even preceding other symptoms. The presence of cold, discolored hands is also frequently seen in MSA. Autonomic dysfunction also occurs in DLB, including orthostatic hypotension and carotid-sinus hypersensitivity causing syncope and transient loss of consciousness. Urinary incontinence also has been reported early in the course of the disease. Urinary incontinence is also seen in PSP patients, although generally not as an early feature. In

contrast, symptoms of dysautonomia in PD are generally mild and are usually absent in the first years of the disease.

Higher cortical dysfunctions

Cognitive dysfunction

This can be seen very early in the course of PD. PD patients generally manifest executive dysfunction, reduced psychomotor speed, visuospatial dysfunction and memory problems. However, the presence of severe cognitive impairment or profound dementia should lead to further investigation, as many of the AP forms (including e.g. PSP, DLB and VaP) demonstrate impaired cognitive function early in the disease course. Apraxia, non-fluent aphasia and behavioral changes can be seen in PSP and especially CBS patients. A particular feature in PSP is a so-called motor recklessness: risky and impulsive behavior, which – in combination with the early and prominent axial disability – commonly leads to severe fall-related injuries. Until recently, it was assumed that cognition remained undisturbed in MSA, in contrary to all other forms of AP. However, recent research has suggested that the cognitive profile of MSA patients is indistinguishable from PSP patients, (Brown et al. 2010) although in our own experience, the clinical impact remains much less pronounced compared to e.g. PSP.

Apraxia

The term apraxia is often used inappropriately in clinical practice. Apraxia formally involves an inability to perform a skilled or learned act that cannot be explained by a language comprehension disorder or elementary motor or sensory deficit. Apraxia is subdivided into three main types: ideomotor apraxia, ideational apraxia and limb kinetic apraxia (table 5). Severe apraxia of all three subtypes can be observed in CBS, causing difficulty in using the afflicted extremity. Characteristically, CBS patients exhibit limb kinetic apraxia prominently and early in the course of the disease. Patients may complain about limited usability of the affected limb, or absent control over movements, and experience an alien limb phenomenon. (Boeve et al. 2003) Limb kinetic apraxia is generally unilateral, mainly involving the most affected limb, as opposed to the other two subtypes. Subtle apraxia, especially involving the eyes, may be seen in PSP patients. In general, apraxia is a red flag for the diagnosis of PD or MSA.

Aphasia

An important discriminating aspect is fluency, which is assessed by asking the patient to name as many animals (category) or words with a specified character (phonemic) in one minute. The presence of aphasia, especially non-fluent aphasia, prompts towards CBS or PSP as the underlying diagnosis. (Boeve et al. 2003; Litvan 2001) However, disturbed fluency is not always due to aphasia, as it can also result from frontal executive dysfunction, which is often seen in (later stages of) PD.

Table 5 Types of apraxia

1. Ideomotor	The patient knows **what** to do, but not **how** to do it; showing difficulty in selection, sequencing and spatial orientation. The patient shows an inability to perform certain tasks on command as well as copying both meaningful and meaningless hand gestures (bilateral). Spontaneous performance of these tasks is remarkably better as well as the performance while using the actual utensil.
2. Ideational	The patient does not know **what** to do. The patient demonstrates a disturbance in the 'design' of complex tasks; disturbance in timing, sequencing and spatial organization of movements. Copying gestures can be remarkably better.
3. Limb kinetic	Disturbance of fine finger movement coordination. The patient demonstrates a unilateral difficulty in copying meaningless hand positions. Mimicking meaningful gestures is generally less troublesome. The use of real objects is typically unimpaired.

Psychiatric symptoms

Apathy is encountered commonly in PD, often together with depression, although it can present separately. In PSP patients, apathy is also common, but usually develops earlier and more prominently compared to PD. Other behavioral changes in PSP include aggressiveness and disinhibition. The latter can be identified using the clapping test: when a patient is asked to clap the hands three times as quickly as possible, PSP patients tend to clap more than the requested three times, and sometimes they cannot stop at all (applause sign). (Dubois et al. 2005) An abnormal clapping test can also occur in PD, so the test does little to help separate PD from AP. Also, emotional disinhibition can occur in PSP and MSA. Hallucinations are a hallmark of Lewy body dementia, especially when these occur prior to administration of dopaminergic therapy. The occurrence of visual hallucinations is associated with greater deficits in cortical acetylcholine, and this might predict a better response to cholinesterase inhibitors. Delusions are also seen frequently in DLB patients, generally of the paranoid type. (McKeith et al. 2005)

Sleeping disturbances

Various sleeping disturbances have been associated with parkinsonian disorders, caused either by the disease itself or by the treatment. Excessive daytime sleepiness is frequently seen in PD and presumably has a multifactorial origin, including poor nighttime sleep and adverse effects of dopaminergic medication. The presence of REM-sleep-behavior-disorder should prompt towards one of the α-synucleopathies (PD, MSA, DLB), although it can be sporadically seen in Alzheimer and other tauopathies (PSP, CBS) as well. Restless legs and periodic limb movements during sleep can be observed frequently in both

PD patients and patients with AP, and presumably have limited discriminative value. The presence of inspiratory stridor suggests a diagnosis of MSA. (Gilman et al. 2008) Because it is a possibly lethal complication that can (partly) be prevented, clinicians should always actively ask for inspiratory stridor, especially if other symptoms suggestive of MSA are present. Validated questionnaires are available to assess different aspects of sleep in PD, including the REM sleep behavior disorder screening questionnaire and the Epworth Sleepiness Scale. (Johns 1991; Stiasny-Kolster et al. 2007)

Response to dopaminergic medication

Patients with PD have a clear and unambiguous levodopa response and tolerate dopaminergic medication well. Most patients with AP react less well or not at all to dopaminergic therapy, even when levodopa is adequately dosed (1000 mg/day during 4 weeks). Approximately one-third of patients with MSA and VP can demonstrate a partial response to levodopa, although a sustained response is rare. Patients suffering from PSP generally do not show a good, sustained response to levodopa. However, an adequate response can be observed in patients with the PSP-P phenotype (where the phenotype is dominated by parkinsonism). Patients with AP tolerate dopaminergic medication less well, and many patients become nauseous when using levodopa. Finally, the presence and nature of dyskinesias can help in the differential diagnosis. Dyskinesias are common in PD, but can be observed in MSA, although the dyskinesias are often localized cranially in MSA, whereas the extremities are usually dyskinetic in PD.

Step 3: The differential diagnosis

The third step is to combine the results of step 1 and 2, as a basis for the differential diagnosis. Table 6 summarizes the clinical signs and symptoms and their presence in the different types of parkinsonism. Interestingly, none of these characteristics or so-called red flags has the ability to fully differentiate between PD and AP. Usually, it is the combination of several atypical features that differentiates both groups, and this often involves pattern recognition (e.g. supranuclear vertical gaze palsy plus motor recklessness; or early autonomic failure with inspiratory stridor). Although such combinations can generally separate the group of PD patients from the group of patients with AP, the overlap between symptom clusters may be too substantial to differentiate at the individual patient level. Therefore, misclassifications still occur even in hands of experts in the field (5-10% negative predictive value in PD). (Hughes et al. 2002)

Table 6 Signs and symptoms for each of the parkinsonisms

	PD	MSA	PSP	CBS	DLB	VaP
Distribution						
- Symmetrical		++	++		+	
- Asymmetrical	+++			+++	+	
- Lower body phenotype						+++
Progression rate						
- Slowly	+++		+		+	
- Quickly		+++	++	++	++	
- Stepwise						+++
Medication						
- Sustained reaction	+++	+	+	-	++	+/-
- Side effects	-	+	+	+	+++	++
Tremor						
- Classical resting tremor	+++	+			+	+
- Jerky tremor	+	+++				+++
Myoclonus	-	+	-	++	+	-
Dysphagia	+	++	+++	+	+	+
Dysarthria	+	+++	+++		+	+
Dystonia	++	++	+	+++		
Pyramidal tract	-	++	-	-	-	+++
Ataxia	-	++	+	-	-	-
Balance disorder						
- Early	-	++	+++			
- Late	++	+++	+++			
Eye movements	+/-	++	+++	+	-	-
Autonomic dysfunction						
- Early	-	+++	-	-	++	+
- Late	++	+++	++	+	++	++
Cognitive dysfunction						
- Early	+/-	-	+	+	+++	++
- Late	++	+/-	++	++	+++	++
Psychiatric problems						
- Hallucinations	-	-	-	-	+++	-
- Depression	++	++				
- Apathy	++	+	++			
- Disinhibition	+/-	++	+++	++		

Table 6 Continued

	PD	MSA	PSP	CBS	DLB	VaP
Sleep disturbances						
- Nighttime stridor		+++				
- RBD	++	++	+/-	+/-	++	-
- RLS/PLM	++	++	++			

PD, Parkinson's disease; MSA, multi system atrophy; PSP, progressive supranuclear palsy; DLB, diffuse Lewy body disease; CBS, corticobasal syndrome; VaP, vascular parkinsonism; RBD, REM-sleep behavior disorder; RLS: restless legs syndrome; PLM, periodic limb movements; -, not present; +++ very often present

The currently used diagnostic consensus criteria for AP (Boeve et al. 2003; Gilman et al. 2008; Litvan et al. 1996; McKeith et al. 2005) have been developed mainly for research purposes, aiming to allocate correctly classified patients to the right studies. These criteria therefore are rather strict, but in daily practice, many patients do not (yet) fully comply with these formal criteria. Yet clinical suspicion can be strong even in such cases, based on the entire clinical picture and own prior experience. We share our clinical suspicion – including the amount of uncertainty – with the patient and family, thus creating transparency.

Two illustrative patient cases

To illustrate the diagnostic process we present two different cases. The first patient, a 58-year old, right-handed man, developed a tremor of his left hand about 12 months ago. His symptoms increased gradually over a period of several months, but remained unilateral. He had not yet been treated with dopaminergic medication. Upon examination, he scored 29/30 points on the MMSE and 18/18 on the FAB (suggesting absence of gross cognitive dysfunction), and 7/39 on the HADS for depression (suggesting absence of depression). The patient denied symptoms suggestive of autonomic dysfunction. Neurological examination shows: a mask-like face, with spontaneously parted lips at rest; asymmetrical bradykinesia, with decrement while performing finger- and foot tapping; a mild rest tremor of the jaw and left hand, including a resetting phenomenon after a postural change; normal range and velocity of eye movements; and absence of ataxia, myoclonus or apraxia. The presence of an asymmetrical hypokinetic-rigid syndrome without red flags renders a diagnosis of PD most likely in this case.

The second patient, a 66-year old right-handed woman, developed difficulty walking about 24 months ago. Over the past years, her symptoms had increased gradually. She developed difficulty maintaining balance, but had never fallen. Just 10 days prior to the

examination, she started using levodopa and reported beneficial effects. She also reported symptoms of orthostatic hypotension and decreased libido, but no urgency or urinary incontinence. Examination revealed a blood pressure of 125/82 without orthostatic hypotension, MMSE 29/30 points, FAB 15/18 (suggesting some frontal executive dysfunction); and 15/39 on the HADS (indicative of depressive symptoms). Neurological assessment further revealed a high-pitched voice, cerebellar ataxia and poliminimyoclonus. Moreover, tandem walking was performed with great difficulty, necessitating a regularly grasping for support. In this patient, a hypokinetic-rigid syndrome was associated with several additional signs that were not particularly helpful for the differential diagnosis (including some frontal executive dysfunction and depression), but also a few clear red flags, including ataxia, polyminimyoclonus and subjective orthostatic hypotension (it can be difficult to prove this using simple sphyngomanometry). (Thijs et al. 2009) This specific combination of symptoms and signs most likely suggests a diagnosis of possible MSA (see table 6).

Conclusion

Establishing a clinical diagnosis of *parkinsonism* can be a challenge, but to differentiate between the different causes of parkinsonism can be even more challenging. Use of a systematic clinical approach, as described in this paper, enables an adequate assessment of the patient, and can help facilitate clinicians in establishing a correct clinical diagnosis early in the course of the disease. However, we do recommend that all patients with a parkinsonian syndrome are referred to a movement disorder expert once in the course of their disease.

3 Biochemistry

3.1 CSF tau protein in the differential diagnosis of parkinsonism, more specific in corticobasal syndrome

Based on

Aerts MB, Esselink RA, Bloem BR, Verbeek MM. Cerebrospinal fluid tau and phosphorylated tau protein are elevated in corticobasal syndrome. Mov Disord. 2011;26(1):169-73.

Summary

Differentiating corticobasal syndrome (CBS) from progressive supranuclear palsy (PSP) and idiopathic Parkinson's disease (PD) can be difficult. To investigate the additional value of cerebrospinal fluid (CSF) biomarkers in the diagnostic differentiation of parkinsonism, we analyzed the CSF concentrations of total protein, lactate and brain specific proteins amyloid-β_{42} protein, tau protein (t-tau), and tau protein phosphorylated at Thr181 (p-tau), in CSF samples from patients with PSP (n =21), CBS (n=12), and PD (n=28). CBS patients demonstrated higher concentrations of t-tau and p-tau compared with PSP and PD patients. In discriminating CBS and PD, t-tau offered the best combination of sensitivity (75%) and specificity (90.9%), followed by p-tau (sensitivity 87.5% and specificity 75%). The p-tau/t-tau ratio resulted in sensitivity of 84.2% and specificity of 66.7% in discriminating PSP and CBS. In conclusion, our results suggest that CSF parameters are of additional value in the diagnostic differentiation of CBS and PD.

Introduction

Corticobasal syndrome (CBS) is an atypical parkinsonian disorder characterized by the combination of cortical dysfunction and extrapyramidal symptoms with profound appendicular dystonia and/or a poor response on levodopa therapy. The cortical dysfunction can comprise a variety of symptoms including the "alien-limb phenomenon". Because Parkinson's disease (PD) and other atypical parkinsonian disorders, like progressive supranuclear palsy (PSP), can closely resemble CBS, the initial diagnosis can be challenging, as reflected by the relatively poor diagnostic accuracy of the clinical diagnosis on neuropathological examination. (Boeve et al. 1999; Boeve et al. 2003; Hughes et al. 2002; Litvan et al. 1997; Wenning et al. 1998)

Corticobasal degeneration is the neuropathological substrate of CBS, and is characterized by the intraneuronal aggregation of tau protein like PSP and Alzheimer's disease (AD), commonly referred to as tauopathies. (Ludolph et al. 2009) In contrast, PD is characterized by α-synuclein inclusions in neurons of the substantia nigra and cortical areas and is therefore classified as an α-synucleinopathy. (Braak et al. 2003)

Currently, the distinction between CBS and other parkinsonian disorders is based mainly on clinical grounds, supported to a limited extent by ancillary investigations. (Abdo et al. 2010) It would be helpful to identify new biomarkers that would facilitate the differential diagnosis of parkinsonism. Due to its proximity to the brain parenchyma, the composition of the cerebrospinal fluid (CSF) may reflect pathologic changes.

Herein, we analyzed the CSF concentrations of Amyloid-β_{42} ($A\beta_{42}$), lactate, total protein, tau protein (t-tau), and tau protein phosphorylated at Thr181 (p-tau), in patients with CBS, PSP, and PD, to investigate the diagnostic ability in differentiating between these parkinsonian syndromes.

Patients and methods

In 2009, a single rater (MBA) retrospectively reassessed the clinical charts of 48 patients, suspected of either PSP or CBS, referred to the Department of Neurology (Radboud University Nijmegen Medical Centre) between 1998 and 2007, who underwent a lumbar puncture during their diagnostic work-up. Reassessment of the clinical diagnosis was performed after a mean 5-years-follow-up period according to international consensus criteria (UK Parkinson's Disease Society Brain Bank clinical diagnostic criteria for PD, (Hughes et al. 1992) Litvan criteria for PSP, (Litvan et al. 1996) Boeve criteria for CBS (Boeve et al. 2003). Diagnostic evaluation included detailed medical history, systematic neurological examination, routine laboratory testing, and a brain magnetic resonance imaging-scan. In addition, many patients underwent neuropsychological assessment, nuclear imaging of cerebral metabolism and/or dopaminergic pathways, electro-oculog-

raphy, and electromyography of the anal sphincter. Only patients diagnosed with either PSP (n=21) or CBS (n=12) and available CSF results were included in this study.
In addition, CSF data of 28 consecutive PD patients, aged older than 50 years and 49 age-matched patients referred to our Neurology Department in that period were analyzed for comparison (reasons for referral: headache (n=15), memory complaints (n=6), functional complaints (n=10), sensory deficits (n=9), dizziness (n=4), and fatigue (n=5)). All controls were diagnosed as not having a neurodegenerative disorder after extensive work-up and had normal routine CSF parameters (normal leukocyte and erythrocyte count, normal protein, glucose and lactate levels, and neither oligoclonal IgG bands nor blood pigments).
The protocols of CSF analysis of $A\beta_{42}$ t-tau, lactate, and total protein were described previously. (Abdo et al. 2004; Abdo et al. 2007) P-tau concentrations in CSF were analyzed using the Innotest Phospho-$Tau_{(181)}$-assay (Innogenetics, Ghent, Belgium; linearity up to 500 ng/L). The interassay variation coefficients over a period of 6 years were 8.8% for t-tau, 6.0% for $A\beta_{42}$, 4.5% for p-tau, and <3% for lactate.
Statistical analysis was performed using GraphPad Prism version 4 (San Diego, CA, USA) and SPSS software version 16.0 (Chicago, IL, USA). Between-groups-analysis was performed using a one-way analysis of variance test or Kruskal-Wallis test for non-normally distributed data. Correction for multiple comparisons was applied. Receiver operator characteristic analysis was used to evaluate the value of individual biochemical variables and their optimal cut-off values discriminating PSP, CBS, and PD. Multivariate logistic regression with backward selection procedures was used to identify variables that contributed independently to discriminate PSP from CBS and CBS from PD.

Results

Twenty-one patients fulfilled the diagnostic criteria of PSP (18 probable PSP, 3 possible PSP; according to the NINDS/SPSP criteria (Litvan et al. 1996), 12 fulfilled the CBS criteria. Thirteen PSP patients and 5 CBS were deceased at the time of the chart review; mean survival was 6.3 years after onset of symptoms in PSP patients and 4.4 years in CBS patients. Neuropathological confirmation of the diagnosis was not available.
Disease severity (H&Y score) at the time of lumbar puncture was significantly higher in PSP patients, when compared with both CBS and PD subgroups. Age, disease duration, and gender distribution were comparable in all 3 groups. Demographic characteristics and CSF parameters are shown in Table 1.
Concentrations of t-tau were significantly higher in CBS patients than in PSP patients, PD patients, and controls ($p<0.001$). P-tau concentrations were significantly higher in the CBS patients, when compared with PSP ($p<0.05$) and PD ($p<0.01$). Lactate concentrations were significantly higher in PSP than in CBS patients ($p<0.05$). $A\beta_{42}$ and total protein concentrations did not differ between PSP and CBS patients. In addition, several ratios

Table 1 Demographic characteristics and results of CSF analysis of the diagnostic groups

Characteristic	PSP	CBS	PD	Controls	*P*-value
Number of patients	21	12	28	49	
Demographic characteristics					
Age (yr)[a]	65.5 (61.0–71.5)	69.0 (62.5–73.0)	62.5 (55.0–69.6)	55.0 (52.0–61.9)	<0.0001[b]
Number of men (%)	10 (48%)	6 (50%)	21 (75%)	26 (53.1%)	NS
Disease duration (yr)[a]	3.0 (1.0–4.0)	2.0 (1.5–4.0)	2.2 (1.7–4.4)	NA	NS
Disease severity, H&Y score[a]	3.0 (3.0–4.0)	2.5 (1.8–3.5)	2.0 (1.5–2.5)	NA	<0.0001[c]
Cognitive function, MMSE score[a]	26.2 (2.5)	21.3 (6.8)	NA	NA	NS
Duration of follow up (yr)	6.1 (3.2)	5.3 (3.1)	7.8 (4.9)	NA	NA
CSF parameters					
T-tau (ng/L)	234 (138)	402 (199)	151 (67.0)	161 (60,7)	<0.0001[d]
P-tau (ng/L)	46.0 (34.5–51.5)	48.0 (38.0–59.0)	46.0 (34.5–51.5)	44.0 (35.5–53.5)	<0.01[e]
$A\beta_{42}$ (ng/L)	704 (181)	730 (316)	744 (201)	838 (253)	NS
Lactate (μmol/L)	1927 (1645–2079)	1666 (1437–1808)	1734 (1478–1941)	1673 (1509–1828)	<0.05[f]
Total protein (mg/L)	584 (157)	488 (126)	511 (129)	475 (135)	<0.05[g]
$A\beta_{42}$/t-tau	3.69 (2.68–4.30)	2.28 (0.64–3.69)	5.79 (3.62–7.68)	5.14 (4.10–6.76)	<0.0001[h]
$A\beta_{42}$/p-tau[a]	15.5 (5.4)	12.9 (7.1)	17.8 (9.7)	18.8 (5.1)	<0.05[i]
P-tau/t-tau	0.21 (0.19–0.27)	0.18 (0.13–0.20)	0.24 (0.22–0.33)	0.26 (0.24–0.32)	<0.05[j]

Data represent median and interquartile range (non-Gaussian distributed data), mean and standard deviation (Gaussian distributed data) or number and percentage. *P* value for differences using 1-way ANOVA. Bonferroni post-hoc test for multiple comparisons was used to identify between-group differences. In cases of non-Gaussian distribution the Kruskal-Wallis test was applied, using Dunn's post-hoc test for multiple comparisons. Gender distribution was analyzed using χ^2 test. PSP, progressive supranuclear palsy; CBS, corticobasal syndrome; PD, Parkinson's disease; H&Y score, Hoehn and Yahr score; MMSE, mini mental state examination; NS, not significant; NA, not assessed.

[a] At the time of lumbar puncture. [b] Controls vs. PSP ($P < 0.001$), vs. CBS ($P < 0.01$) and vs. PD ($P < 0.05$). [c] PSP vs. PD ($P < 0.001$). [d] CBS vs. controls, PSP and PD ($P < 0.001$). PSP vs. PD and controls ($P < 0.05$). [e] CBS vs. controls ($P < 0.01$), and vs. PD ($P < 0.05$). [f] PSP vs. CBS ($P < 0.05$). [g] PSP vs. controls ($P < 0.05$). [h] CBS vs. PD and controls ($P < 0.001$). PSP vs. controls ($P < 0.05$). [i] Between group difference not significant after correction for multiple comparisons. [j] CBS vs. controls ($P < 0.05$).

were calculated. As expected, the $A\beta_{42}$/t-tau ratio differed significantly between PSP and CBS (Table 1).

We neither established correlations between CSF parameters and age, disease duration, nor severity. However, mini-mental state examination (MMSE) scores correlated with $A\beta_{42}$ (r=0.481, $p<0.05$), t-tau (r=−0.622, $p=0.002$), and p-tau (r= −0.642, $p=0.001$) in the CBS subgroup. Similar results were obtained in the combined CBS and PSP subgroups.

Univariate logistic regression analysis was carried out to discriminate CBS from PSP revealing that neither sensitivity nor specificity exceeded 80% for individual parameters (Table 2). Therefore, multivariate logistic regression analysis was carried out to improve

Table 2 ROC-analysis of CSF analysis for CBS vs. either PSP or PD

CSF variables	Cut-off	Sens	Spec	AUC (95% CI)	LR[a]
PSP vs. CBS					
Univariate					
T-tau (ng/L)	>322	80.0%	63.6%	0.77 (0.61–0.95)	2.20
P-tau (ng/L)	>52	63.2%	75.0%	0.76 (0.59–0.93)	2.53
$A\beta_{42}$ (ng/L)	<655	73.7%	50.0%	0.53 (0.30–0.76)	1.47
Lactate (µmol/L)	<1769	63.2%	80.0%	0.74 (0.55–0.93)	3.16
Total protein (mg/L)	<539	63.2%	72.7%	0.68 (0.48–0.88)	2.32
$A\beta_{42}$/t-tau	<3.22	68.4%	75.0%	0.72 (0.52–0.92)	2.74
$A\beta_{42}$/p-tau	<7.76	94.7%	41.7%	0.64 (0.42–0.85)	1.62
P-tau/t-tau	<0.18	84.2%	66.7%	0.75 (0.56–0.93)	2.53
Multivariate					
Model 1[b]	<3.95	93.8%	70.0%	0.89 (0.77–1.00)	3.13
Model 2[c]	<1.91	68.8 %	90.0%	0.88 (0.75–1.00)	6.88
CBS vs. PD					
Univariate					
T-tau (ng/L)	>197	75.0%	90.9%	0.91 (0.82–1.00)	8.24
P-tau (ng/L)	>52.5	87.5%	75.0%	0.80 (0.64–0.97)	3.50
$A\beta_{42}$ (ng/L)	<658	75.0%	50.0%	0.56 (0.34–0.77)	1.50
$A\beta_{42}$/t-tau	<3.21	85.7%	75.0%	0.86 (0.74–0.98)	3.43

[a] Likelihood ratio: sensitivity/(1 − specificity)

[b] Model 1: $Y = -1.539 + (0.002 \times \text{tau}) + (0.003 \times \text{lactate}) + (-0.098 \times \text{p-tau}) + (0.004 \times \text{protein})$

[c] Model 2: $Y = 0.122 + (0.003 \times \text{lactate}) + (-0.081 \times \text{p-tau})$

ROC-analysis, receiver operating characteristic analysis; PSP, progressive supranuclear palsy; CBS, corticobasal syndrome; PD, Parkinson's disease; CSF, cerebrospinal fluid; t-tau, total tau protein; p-tau, phosphorylated tau protein; $A\beta_{42}$, amyloid-β_{42} protein; sens: sensitivity; spec: specificity; AUC: area under the curve; 95 CI, 95% confidence interval; LR: likelihood ratio.

diagnostic accuracy in discriminating between CBS and PSP. T-tau, p-tau, lactate, and total protein concentrations were added to the selection procedure. The prediction model thus constructed reached a sensitivity of 93.8% and a specificity of 70.0%. A prediction model based on only p-tau and lactate reached a sensitivity of 68.8% and a specificity of 90.0%.

Univariate analysis demonstrated that t-tau protein offered the best combination of sensitivity (75%) and specificity (90.9%) to differentiate between CBS and PD. The concentration of p-tau showed a sensitivity of 87.5% and a specificity of 75%. Multivariate logistic regression analysis was performed, selecting only t-tau protein as independent marker to separate CBS and PD.

Discussion

This study is one of the few to compare CSF biomarkers in patients with CBS, PSP, and PD with extended clinical follow-up. We demonstrated that the concentrations of t-tau and p-tau proteins in CSF of CBS patients were significantly elevated compared with PSP and PD. However, the diagnostic accuracy of CSF t-tau and/or p-tau seems only sufficient in the discrimination of CBS vs. PD not in discriminating CBS vs. PSP.

As the sensitivity for the initial clinical diagnosis CBS is poor, the CSF profile of CBS (i.e., increased t-tau and p-tau) may increase awareness for the diagnosis CBS. A timely and correct diagnosis may result in better targeted treatment strategies, adequate patient counseling and —perhaps most important— early recognition of disease-specific complications.

In AD $A\beta_{42}$, CSF concentrations are decreased, (Wiltfang et al. 2005) whereas in parkinsonian disorders, the data seem conflicting, (Compta et al. 2009; Holmberg et al. 2003; Verbeek et al. 2004) presumably due to large inter-individual variability, underpowered studies, and possibly different underlying pathology as neuropathological confirmation is lacking in most studies. (Arai et al. 1997; Borroni et al. 2008; Mitani et al. 1998; Mollenhauer et al. 2008; Noguchi et al. 2005; Urakami et al. 2001) T-tau and p-tau concentrations are reported to be increased in tauopathies, predominantly AD, (Hampel and Teipel 2004) but also—in line with our results—in CBS compared with controls. (Borroni et al. 2008; Mitani et al. 1998; Mollenhauer et al. 2008) However, other studies failed to demonstrate such elevations in CBS, (Arai et al. 1997; Noguchi et al. 2005) a disparity possibly caused by enrolment of more severely cognitively affected patients in our study, possibly reflecting more cortical involvement correlating with higher CSF t-tau levels (mean MMSE score 21.3 vs. 28.0). (Noguchi et al. 2005)

Although both PSP and CBS are neuropathologically characterized by axonal degeneration and the accumulation of t-tau protein in the brain, only in CBS this seems to lead to an increase in CSF t-tau and p-tau concentrations. (Arai et al. 1997; Borroni et al. 2008; Noguchi

et al. 2005; Paraskevas et al. 2005; Urakami et al. 2001) The observed difference between CBS and PSP may be explained by a higher rate of atrophy, a larger brain area involved and relative proximity of the affected brain areas to the CSF compartment in CBS. (Boxer et al. 2006; Groschel et al. 2004; Schofield et al. 2005; Whitwell et al. 2007) Interestingly, elevated concentrations of t-tau protein were observed previously in MSA, an α-synucleinopathy, stroke, and Creutzfeldt-Jakob disease. (Abdo et al. 2004; Abdo et al. 2007; Hesse et al. 2000) Hence, t-tau concentrations might be a biomarker for accelerated degeneration instead of reflecting the pathological substrate.

This study has several drawbacks. First, due to the retrospective character of the study selection bias cannot be ruled out, because only patients with diagnostic uncertainty were included in this study, possibly leading to the selection of more atypical phenotypes. However, the studied population therefore closely resembles daily clinical practice in which ancillary diagnostic tests are applied in cases of diagnostic uncertainty. Second, the clinical diagnosis was not confirmed neuropathologically and therefore susceptible to misclassification. However, the final diagnosis was based on thorough clinical and ancillary investigations (including nuclear imaging and neuropsychological assessment), after extensive follow-up and according to international consensus criteria in a specialized movement disorder clinic. In addition, charts of patients with CSF parameters in overlapping ranges or with Alzheimer-like CSF profiles were re-examined to assess whether these patients exhibited clinical features suggesting potential misdiagnosis with PSP, CBS, or AD; no misdiagnosed cases were identified.

In conclusion, despite these drawbacks, our results suggest that CSF analysis could aid the diagnostic differentiation of CBS and PD. Abnormal CSF t-tau and p-tau concentrations may raise awareness for the diagnosis CBS. These results warrant validation in a prospective study with neuropathological confirmation of the diagnosis.

3.2 CSF alpha synuclein in the differential diagnosis of parkinsonism

Based on

Aerts MB, Esselink RA, Abdo WF, Bloem BR, Verbeek MM. CSF α-synuclein does not differentiate between parkinsonian disorders. Neurobiol Aging. 2012; 33(2):430.e1-3.

Summary

Differentiating between Parkinson's disease (PD) and atypical parkinsonism (AP) is clinically relevant but challenging. A timely and correct diagnosis might result in better targeted treatment strategies, adequate patient counseling, and early recognition of disease-specific complications. We aimed to investigate whether cerebrospinal fluid (CSF) concentrations of α-synuclein are of additional diagnostic value.

We examined 142 consecutive patients with parkinsonism, mean disease duration 39.7 months. (PD, n=58; MSA, n=47; DLB, n=3; VaP, n=22; PSP, n=10; CBS, n=2) Gold standard was the clinical diagnosis established after three years of clinical follow-up. CSF concentrations of α-synuclein, blood pigments and the erythrocyte count were determined.

No differences between CSF α-synuclein concentrations of patients with PD with the reference values from our laboratory were observed. We neither found significant differences between patients with PD and AP nor between AP subgroups. Adjustment for age, disease severity or presence of erythrocytes or blood pigments in CSF did not alter these results.

Our results imply that CSF α-synuclein is currently unsuitable as biomarker to differentiate between PD and AP.

Introduction

Idiopathic Parkinson's disease (PD) is the most common neurodegenerative movement disorder, clinically characterized by the classical triad of bradykinesia, tremor and rigidity. (Daniel and Lees 1993) The initial diagnosis can be challenging, since so called 'atypical parkinsonian disorders', like multiple system atrophy (MSA) can closely resemble PD, especially in the early phases of disease. This challenge is reflected in the clinico-pathological observation that upon post mortem evaluation out of the patients wrongly diagnosed with MSA, 80% turned out to suffer from PD instead of MSA. (Hughes et al. 2002)

The differentiation between PD and atypical parkinsonism is highly important. A correct diagnosis might result in better targeted treatment strategies, more adequate patient counseling and –perhaps most important- timely recognition of disease specific symptoms like nighttime stridor, early dysphagia, or postural hypotension to prevent complications like aspiration or falls in MSA.

PD is neuropathologically characterized by α-synuclein inclusions, so called Lewy bodies, in dopaminergic neurons of the substantia nigra and cortical areas and is therefore classified as an α-synucleinopathy. Alpha synuclein is a natively unfolded, soluble protein, abundantly present in presynaptic nerve termini. Its precise function is still unknown. (Uversky 2007) Alpha synuclein may adopt a β-sheet conformation leading to its self-aggregation, but the mechanisms leading to the formation of these Lewy bodies are not fully elucidated yet. Presumably a combination of the propensity of self aggregation of the α-synuclein protein, α-synuclein mutations and multiplication of the coding gene and post-translational modifications such as phosphorylation or ubiquitination, promote the formation of the characteristic Lewy bodies. (Mukaetova-Ladinska and McKeith 2006)

The group of atypical parkinsonisms is neuropathologically heterogeneous. Vascular parkinsonism (VaP) is presumably caused by white matter lesions in predominantly frontal and thalamic regions, and/or strategic lesions in the basal ganglia. (Zijlmans et al. 2004) Progressive supranuclear palsy (PSP) and corticobasal syndrome (CBS) are characterized by intraneuronal deposition of the tau protein, and are therefore part of the spectrum of tauopathies that also includes Alzheimer's disease. (Hampel and Teipel 2004) Dementia with Lewy bodies (DLB) and MSA are also characterized by α-synuclein inclusions and are therefore, like PD, classified as α-synucleinopathies.

Since cerebrospinal fluid (CSF) directly communicates with the extracellular fluid surrounding brain cells, the CSF α-synuclein concentration may reflect the neuropathological changes observed in α-synucleinopathies and tauopathies. Interestingly, a recently published study indeed showed markedly lower levels of α-synuclein in the CSF of PD patients as compared to controls. (Hong et al. 2010) The potential use as a biomarker of α-synuclein in the differentiation between different parkinsonian disorder has, however, not rigorously been studied yet. In this study, we aimed to investigate the value of

α-synuclein as a biomarker for the differentiation of parkinsonian syndromes in a large cohort of consecutive patients, prospectively followed-up for three years.

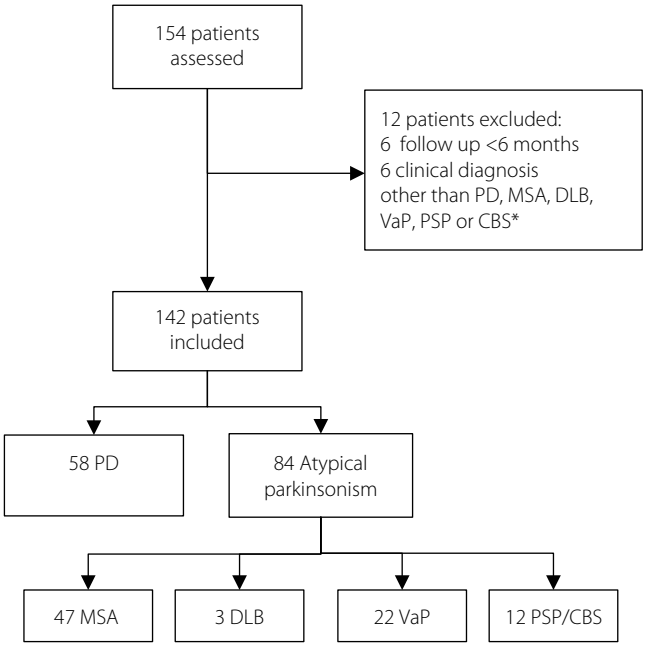

Figure 1 Flow chart.

PD: Parkinson's Disease; MSA: multisystem atrophy; DLB: dementia with Lewy bodies; VaP: vascular parkinsonism; PSP: progressive supranuclear palsy; CBS: corticobasal syndrome

* This group included 1 patient with dystonic tremor, 1 patient with neuroborreliosis, 1 patient with hydrocephalus and essential tremor, 1 patient with amyotrophic lateral sclerosis and 2 patients with psychogenic complaints.

Methods

Patients

Between January 2003 and December 2006 all patients with a hypokinetic rigid syndrome in which diagnostic uncertainty existed regarding the underlying etiology, referred to our center were asked to participate in this study. The full studyprotocol is described in chapter 4. The patients included in the current study are a subset of the large study described in chapters 4 and 5. This subset of patients contains all included patients who underwent a lumbar puncture. Patients also underwent an MRI, IBZM-SPECT scan and electromyography of the anal sphincter according to study protocol. Patients were re-examined after a follow-up period of 3 years.

Disease severity at the time of lumbar puncture was established according to the (modified) Hoehn and Yahr stages (H&Y) (Hoehn and Yahr 2001) and Unified Parkinson's Disease Rating Scale (UPDRS). Cognitive function was assessed using the Mini Mental State Examination (MMSE). (Folstein et al. 1983)

Clinicopathological data have shown a high concordance between the neuropathological and clinical diagnosis after substantial duration of follow up (at least 2 years) by a movement disorder specialist. (Hughes et al. 2002) We therefore used the final clinical diagnosis as gold standard in our study, which was established by consensus between two experienced movement disorder specialists (BB and RE) who were blinded to the CSF results, and always according to established diagnostic clinical criteria for PD, (Gelb et al. 1999) MSA, (Gilman et al. 2008) PSP, (Litvan et al. 1996) DLB, (McKeith et al. 2005) CBS, (Boeve et al. 2003) and VaP. (Zijlmans et al. 2004)

To determine reference values for the various CSF markers in a non-parkinsonian population, we selected age-matched controls who were referred to our Neurology Department between 2004 and 2007 and who had a lumbar puncture as part of a diagnostic work-up. All controls were diagnosed as not having a neurodegenerative disorder after extensive work-up, and had normal routine CSF parameters (normal leukocyte and erythrocyte count, normal protein, glucose and lactate levels and neither oligoclonal IgG bands nor blood pigments). These data have been published previously (Spies et al. 2009) Medical ethical approval for this study was obtained from the institutional review board. Written informed consent and approval by the local ethical committee were obtained.

CSF analysis

CSF samples were collected in polypropylene tubes, centrifuged, aliquoted, and stored at -80°C until analysis. The following CSF variables were taken into account for the present study: α-synuclein, blood pigments and the total erythrocyte count. The erythrocyte count and blood pigments were analyzed within 2 hours after CSF collection, α-synuclein was analyzed in the entire group at once to minimize inter-assay variability. The method of α-synuclein analysis and its validation have been published previously. (van Geel et al. 2008) The linearity of the used assay ranged from 6 to 300ng/ml. The intra-assay coefficient of variation was 3.5% at a concentration of 49ng/ml. The number of erythrocytes was manually counted in a counting chamber (volume 3 µl), blood pigments were analyzed using spectrophotometric analysis of centrifuged CSF (Perkin-Elmer, Groningen; The Netherlands) and calculation of the second derivative of the signal (which is linear with the concentration) in the spectrum between 573 and 578 nm for hemoglobin and the spectrum between 460 and 478 nm for bilirubin.

Statistical analysis

The χ^2 test was used in case of 5x2 contingency tables. Between-groups analysis was performed using a one-way ANOVA test, or a student's t-test in case of 2 groups in case of

normally distributed data. Non-normally distributed data were analyzed using a Kruskal-Wallis test or Mann-Whitney test in case of 2 groups. Pearson correlation coefficient was used to analyze correlations. Covariates were examined by linear regression (ANCOVA). Prior to correlation and covariate analysis, non-normally distributed data were log transformed to meet assumptions of normality and homogeneity. Statistical analysis was carried out using GraphPad Prism version 4 (San Diego, CA) and SPSS software version 16.0 (Chicago, IL).

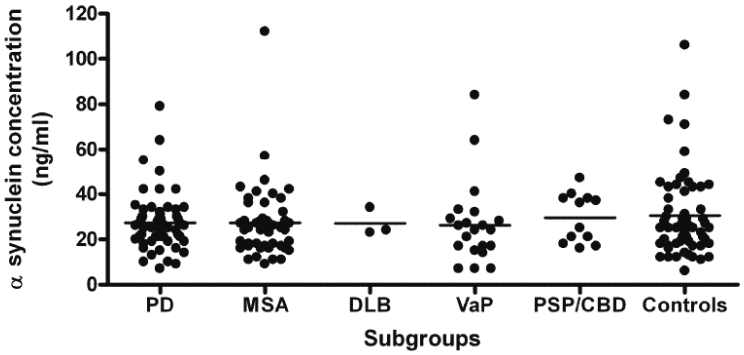

Figure 2 Scatterplots of the CSF concentrations of α-synuclein (ng/L) in PD, MSA, DLB, VaP, PSP/ CBS and control subgroups.

Horizontal lines represent mean levels. PD: Parkinson's Disease; MSA: multisystem atrophy; DLB: dementia with Lewy bodies; VaP: vascular parkinsonism; PSP: progressive supranuclear palsy; CBS: corticobasal syndrome

Results

Patients

A total of 142 consecutive patients were included in this study between August 2003 and December 2006. (Figure 1) Fifty-eight patients had a final clinical diagnosis of PD, 47 of multisystem atrophy (MSA) (25 possible, 20 probable and 2 definite MSA), 3 of dementia with Lewy bodies (DLB) (2 possible, 1 probable DLB), 22 of vascular parkinsonism, (VaP), 10 of progressive supranuclear palsy (PSP) (6 possible, 3 probable and 1 definite PSP) and 2 of corticobasal syndrome (CBS). In 43 out of these 142 patients we were not able to complete the 2-years follow-up for the following reasons: 16 patients had deceased prior to completion of the entire follow up, neuropathological confirmation of the diagnosis was available in 3 of these cases (2 MSA, 1 PSP); 12 patients were too severely affected by the disease to enable follow up, and 15 patients were lost to follow up or declined to participate to the follow-up session. Chart review over the maximum available follow-up period

was used for the final diagnosis in these 39 cases and the neuropathological diagnosis was used in 4 cases. The mean follow-up period for the patients lost to follow-up was 18.8 months compared to 36 months in the patients that were not lost to follow-up. For the purpose of analysis, the PSP and CBS patients were combined. Demographic characteristics are shown in Table 1.

Age at the time of lumbar puncture and disease severity (measured by UPDRS score as well as Hoehn and Yahr score) were significantly lower in the patients with PD as compared to the patients with VaP. Disease duration however, was comparable in all subgroups. No significant differences in cognitive function were observed between subgroups.

CSF analysis

The results of the CSF analysis are presented in Table 2 and Figure 2.

CSF α-synuclein data were log-transformed to meet assumptions of normality and homogeneity. We observed no differences in CSF α-synuclein concentrations between patients with PD and healthy controls. We neither observed any differences in α-synuclein concentrations between the PD group and the group of atypical parkinsonism, nor between the different subgroups of atypical parkinsonism. Furthermore, we did not observe any differences in α-synuclein concentration when the group of α-synucleinopathies (PD, MSA and DLB) was compared with the group of tauopathies (PSP and CBS). Analyzing patients with only probable or definite diagnosis, and analyzing only patients who completed the entire follow up duration did not change our results.

Correlation analysis revealed a weak correlation between both the erythrocyte count and the hemoglobin concentrations in CSF on the one hand and the α-synuclein concentrations on the other ($r=0.201$ and 0.191, $p<0.05$) in the entire group of patients. These correlations were not present in any of the subgroups.

Within the PD group, α-synuclein concentrations were negatively correlated with age at time of lumbar puncture ($r=-0.358$, $p<0.006$). This correlation however, could not be established within other subgroups or within the entire group of patients. Within the MSA subgroup, α-synuclein concentrations were correlated with Hoehn and Yahr scores ($r=0.409$, $p<0.004$). The correlation was not found in the entire patient group or in the other subgroups. No correlations between α-synuclein concentrations and cognitive function (measured by MMSE) could be established, neither within subgroups, nor within the entire group of patients.

Despite the aforementioned correlations, no differences in α-synuclein concentrations could be observed between PD patients and controls or between the various patients groups if only patients with a normal free hemoglobin (≤0.25 μM) and erythrocyte count (≤50/μl) were included, or when α-synuclein concentrations were adjusted for age or disease severity.

Table 1 Demographic characteristics of the diagnostic groups

Characteristic	PD	MSA	DLB	VaP	PSP/CBS	Controls	p-value[b]
Number of patients	58	47	3	22	12	57	
Age (yrs)[a]	56.6 (10.8)	62.9 (7.8)	62.5 (6.9)	68.7 (6.5)	66.2 (8.8)	61.3 (8.8)	<0.001[c]
Number of men (%)	40 (69%)	28 (60%)	3 (100%)	15 (67%)	5 (42%)	30 (53%)	NS (p=0.20)
Disease duration (months)[a]	37.4 (32.6)	38.5 (27.7)	60.0 (20.8)	46.9 (31.2)	36.8 (20.0)	NA	NS (p=0.22)
Disease severity (H&Y score)[a]	1.8 (0.6)	2.3 (1.0)	2.7 (0.6)	2.8 (0.9)	2.8 (0.7)	NA	<0.001[d]
Disease severity (UPDRS score)[a]	24.3 (11.9)	29.5 (14.3)	34.7 (10.4)	36.7 (12.4)	30.8 (12.1)	NA	<0.01[e]
Cognitive function (MMSE)[a]	28.4 (1.6)	27.9 (2.5)	22.7 (6.4)	26.6 (2.7)	27.2 (1.8)	NA	NS (p=0.02)[f]

Mean values and standard deviations are shown. PD, Parkinson's disease; MSA, multiple system atrophy; DLB, dementia with Lewy Bodies; VaP, vascular parkinsonism; PSP, progressive supranuclear palsy; CBS, corticobasal syndrome; H&Y score, Hoehn and Yahr score; UPDRS score, unified Parkinson's disease rating scale; MMSE, mini mental state examination; NS, non significant; NA, not applicable

[a] At the time of lumbar puncture

[b] p-value for differences using the Kruskal-Wallis test with Dunn's post-hoc test for multiple comparisons was used to identify between-group differences. Gender distribution was analyzed using χ^2 test.

[c] p-value for PD vs. VaP p<0.05 for PD vs. PSP/CBS and for VaP vs. controls. No significant difference between the other subgroups.

[d] p-value for PD vs. VaP and PD vs. MSA. P<0.01 for PD vs. PSP/CBS. No significant difference between the other subgroups.

[e] p-value for PD vs. VaP. No significant difference between the other subgroups

[f] Significance is lost when correcting for multiple comparisons

Table 2 CSF parameters by diagnostic group

CSF parameters	PD (n=58)	MSA (n=47)	DLB (n=3)	VaP (n=22)	PSP/CBS (n=12)	Controls (n=57)	p-value[a]
α-synuclein (ng/ml)[b]	26.0 (20.5-32.5)	25.0 (17.0-32.0)	24.0 (23.0-34.0)	24.0 (16.0-30.5)	30.5 (19.5-38.0)	25.0 (18.0-42.0)	NS (p=0.79)
Total erythrocyte count (number/µl)[b]	1 (0-16)	3 (0-62)	21 (0-23)	3.5 (0-38)	2.5 (0.5-23.5)	Normal[c]	NS (p= 0.50)
Hemoglobin (µmol/l)	0.017 (0.05)	0.046 (0.10)	0 (0)	0.032 (0.06)	0.003 (0.01)	Normal[d]	NS (p= 0.29)
Bilirubin (µmol/l)	0.031 (0.13)	0.029 (0.07)	0 (0)	0.024 (0.06)	0.014 (0.05)	Normal[e]	NS (p= 0.55)

Mean values and standard deviations are shown, unless otherwise specified. PD, idiopathic Parkinson's disease; MSA, multiple system atrophy; DLB, dementia with Lewy Bodies; VaP, vascular parkinsonism; PSP, progressive supranuclear palsy; CBS, corticobasal syndrome; NS, non significant.

[a] p-value for differences using 1-way ANOVA or the Kruskal-Wallis test to identify between-group differences.

[b] Median and p25-p75 ranges are shown.

[c] Normal≤ 50/µl

[d] Normal ≤0.25 µmol /l

[e] Normal ≤0.50 µmol /l

Discussion

Our study is the first to assess the value of CSF α-synuclein concentrations in differentiating between PD and AP. Despite the large sample-size, we found no differences in α-synuclein concentrations between different patient groups. Furthermore, we could not replicate the previously reported decrease in CSF α-synuclein concentration in patients with PD as compared to controls. (Hong et al. 2010) Moreover, these findings suggest that α-synuclein has no value as biomarker for the differential diagnosis of parkinsonian syndrome.

The comparability of different studies describing CSF α-synuclein levels is somewhat hindered by the use of different methods to measure CSF α-synuclein concentrations and the use of different antibodies in the respective assays to detect α-synuclein. Possibly as a result, previous studies have shown contradicting results. In a recent study, α-synuclein concentrations were decreased in PD patients as compared to controls - in line with previous studies-, (Mollenhauer et al. 2008; Tokuda et al. 2006) whereas others, including our study, did not find significant differences. (Borghi et al. 2000; Ohrfelt et al. 2009) Besides different methods for detection, the observed disparity could partly be explained by differences in the selection of control populations, due to age effects and other possible confounders like coexisting neurological diseases. Furthermore, if any, the observed differences were small, (Hong et al. 2010; Mollenhauer et al. 2008; Tokuda et al. 2006) with profound overlap between the different patients groups, resulting in insufficient sensitivity and specificity numbers to warrant the application of CSF α-synuclein analysis in daily practice.

Currently, 4 isoforms of α-synuclein are known. (Beyer et al. 2008) Isoform α-synuclein-140 is the best known isoform and comprises the whole transcript of the protein. The other 3 isoforms, α-synuclein-126, α-synuclein-112 and α-synuclein-98, are the result of alternative splicing causing in-frame deletions of exon 3 (amino acids 41-54) and exon 5 (103-130) and both exon 3 and 5 respectively. (Beyer et al. 2008; Uversky 2007) Especially the deletion of exon 5 might prove highly relevant, since the majority of the antibodies used for the detection of α-synuclein are directed against these residues. (Hong et al. 2010; Mollenhauer et al. 2008; Tokuda et al. 2006) Therefore, both α-synuclein-112 and α-synuclein-98 isoforms are not routinely measured in CSF. Interestingly, differences in expression of these isoforms have been described in both PD and DLB patients. (Beyer et al. 2008) Perhaps the profile of all 4 isoforms, instead of only the concentration of exon 5 -containing α-synuclein protein, would distinguish between different neurodegenerative diseases. Hence, further research should focus on the development and validation of antibodies targeted against the specific isoforms, in addition to the presently available assays to detect full-length α-synuclein protein.

The weak negative correlation observed between α-synuclein concentration and age in PD is concurrent with previous studies (Hong et al. 2010; Spies et al. 2009; Tokuda et al. 2006) and is possibly caused by age-depended changes in the velocity of axonal transport

of α-synuclein and decreasing availability of soluble α-synuclein in the aging brain. (Mukaetova-Ladinska and McKeith 2006)

The weak correlation between α-synuclein and free hemoglobin or total erythrocyte count has been described before. (Barbour et al. 2008; Hong et al. 2010) Alpha-synuclein in blood is present in red blood cells (RBC) and considering the fragility of RBCs, α-synuclein levels in CSF may be artificially elevated by CSF contamination with RBCs. (Barbour et al. 2008) Hence, we also analyzed the subgroup of patients without detectable blood pigments in CSF and with erythrocyte counts below 50/μl, but reached similar conclusions.

We did observe a correlation between disease severity (as measured by H&Y score) and α-synuclein concentration in the MSA subgroup, as opposed to previous research. (Tokuda et al. 2006) Possibly the increased rate of disease progression in MSA and resulting cell death cause a release of the presynaptically localized α-synuclein, thus influencing the CSF α-synuclein concentration slightly.

The prevalence of PSP and CBS in our study population was rather low. As a result, the group of atypical parkinsonism in our study consisted mainly of MSA patients. Since both MSA and PD are neuropathologically characterized as α-synucleinopathies, the conducted analyses might have been dominated by the overrepresentation of α-synucleinopathies. Possibly a more balanced inclusion of both patients with an α-synucleinopathy and patients with a tauopathy might lead to different observations.

In our specialized movement disorder center, referral and selection bias may have prompted the inclusion of an atypical patient population, which may have lead to more diagnostic uncertainty. Possibly this adds to the observed differences between our and previous studies. However, our study population therefore closely resembles the daily clinical practice in which ancillary diagnostic tests are applied in cases of diagnostic uncertainty. In addition, the clinical diagnosis was confirmed neuropathologically in only 3 cases, and therefore potentially susceptible to misclassification. However, our gold standard diagnosis was based on careful clinical assessment at baseline and after 2 years of clinical follow-up, including response to treatment and baseline cerebral MRI in all patients. For most patients, a proper diagnosis can be made after two years of follow-up and treatment with an adequate dose of levodopa. Half of the included patients were diagnosed according to the definite or probable diagnostic criteria. Moreover, separate analysis of CSF α-synuclein including only the patients with either a probable or definite diagnosis according to the current criteria did not yield different results, and neither did subgroup analysis including only patients who completed the entire follow up duration.

In conclusion, despite the aforementioned drawbacks, our results imply that CSF α-synuclein is currently not advocated as a diagnostic biomarker in differentiating between PD and atypical parkinsonism.

3.3 CSF neurotransmitters in the differential diagnosis of dementia

Based on

CSF tau, Aβ42, and MHPG differentiate dementia with Lewy bodies from Alzheimer's disease. Aerts MB, Esselink RA, Claassen JA, Abdo WF, Bloem BR, Verbeek MM. J Alzheimers Dis. 2011;27(2):377-84.

Summary

Differentiating dementia with Lewy bodies (DLB) from Alzheimer's Disease (AD) can be difficult because of the substantial overlap in clinical features. Since deficits in serotonergic and dopaminergic pathways seem more pronounced in DLB patients, we investigated whether cerebrospinal fluid (CSF) analysis of neurotransmitter metabolites, additional to brain-specific proteins, may improve the differentiation between DLB and AD.

We retrospectively compared CSF concentrations of the neurotransmitter metabolites homovanillic acid (HVA), 5-hydroxyindolacetic acid (5-HIAA) and 3-methoxy-4-hydroxy-phenylethyleneglycol (MHPG) and the brain-specific proteins total tau (t-tau), phosphorylated tau protein (p-tau) and amyloid-β_{42} ($A\beta_{42}$) in 45 patients with AD (mean age 71.6 years; 34 (76%) men; 44 probable AD, 1 definite) and 23 patients with DLB (mean age 71.6 years; 18 (78%) men; 6 possible DLB, 16 probable, 1 definite).

The concentrations of all neurotransmitter metabolites, as well as those for t-tau and p-tau protein, were significantly lower in DLB compared to AD, irrespective of the diagnostic certainty (i.e. possible or probable). The currently used combination of $A\beta_{42}$, p-tau and t-tau yielded a sensitivity of 92.9% and a specificity of 90%. The addition of MHPG to resulted in an increased sensitivity of 97.6% and a specificity of 95% for the discrimination between DLB and AD.

In conclusion, the combination of MHPG and the brain specific proteins t-tau, p-tau and $A\beta_{42}$ in CSF were associated with the clinical diagnosis of DLB and discriminated between AD and DLB with high diagnostic accuracy, suggesting this combination as a potential biomarker for DLB.

Introduction

Dementia with Lewy bodies (DLB) is a common cause of dementia, accounting for up to 20% of the dementia population. (Rahkonen et al. 2003) Core features include fluctuating cognition, visual hallucinations, autonomic disturbances and parkinsonism. The neuropathological hallmark is the 'Lewy body', an intraneuronal inclusion body consisting of, amongst other, ubiquitin and α-synuclein, present in the substantia nigra, neocortex as well as limbic and forebrain structures. (D. F. Brown 1999; I. McKeith et al. 2004)
Differentiation between DLB and Alzheimer's disease (AD) on clinical grounds alone can be difficult due to substantial overlap in clinical presentation, as reflected by low sensitivity of the clinical criteria for DLB. (Litvan et al. 2003; I. McKeith et al. 2004; Nelson et al. 2010) However, early recognition is important because of the therapeutic consequences. DLB patients show great sensitivity to neuroleptics, which may cause physical and cognitive deterioration, and even increased mortality. (Aarsland et al. 2005; Henriksen et al. 2006; I. McKeith et al. 1992) Hence, biomarkers that improve the early recognition of DLB are urgently needed.
Currently the cerebrospinal fluid (CSF) concentrations of amyloid-β_{42} ($A\beta_{42}$), total tau protein (t-tau) and tau protein phosphorylated at Thr181 (p-tau) are employed to identify (incipient) AD patients amongst patients with dementia syndromes or MCI. (Mattsson et al. 2009) However, the differentiation between the different dementia syndromes based on CSF analysis is limited, e.g. because of considerable overlap between AD and DLB. (Andreasen et al. 2001; Bibl et al. 2006; Clark et al. 2003; Gomez-Tortosa et al. 2003; Kanemaru et al. 2000; Tschampa et al. 2001; Verbeek et al. 2003)
Deficits in serotonergic and dopaminergic pathways, associated with symptoms of autonomic dysfunction or parkinsonism, are more pronounced in DLB patients compared to AD patients. (Klein et al. 2010; Perry et al. 1991; Walker et al. 2002; Walker et al. 2007) We anticipated that CSF concentrations of the neurotransmitter metabolites homovanillic acid (HVA), 5-hydroxyindolacetic acid (5-HIAA) and 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG), the catabolic end-products of dopamine and epinephrine degradation, are lower in DLB than in AD. (Kanemaru and Yamanouchi 2002; Langlais et al. 1993; Perry et al. 1993; Weiner et al. 1996) Therefore, we aimed to investigate the additional diagnostic value of CSF neurotransmitter metabolites compared to CSF brain-specific proteins in differentiating between DLB and AD.

Methods and Materials

Patients

We included consecutive patients with a clinical diagnosis of DLB who were referred to either the movement disorder clinic of the Department of Neurology or the memory

clinic of the Department of Geriatric Medicine at the Radboud University Nijmegen Medical Centre -to cover the broad clinical spectrum of DLB-, who underwent a lumbar puncture between December 2003 and June 2008 as part of the diagnostic work-up. (Figure 1)

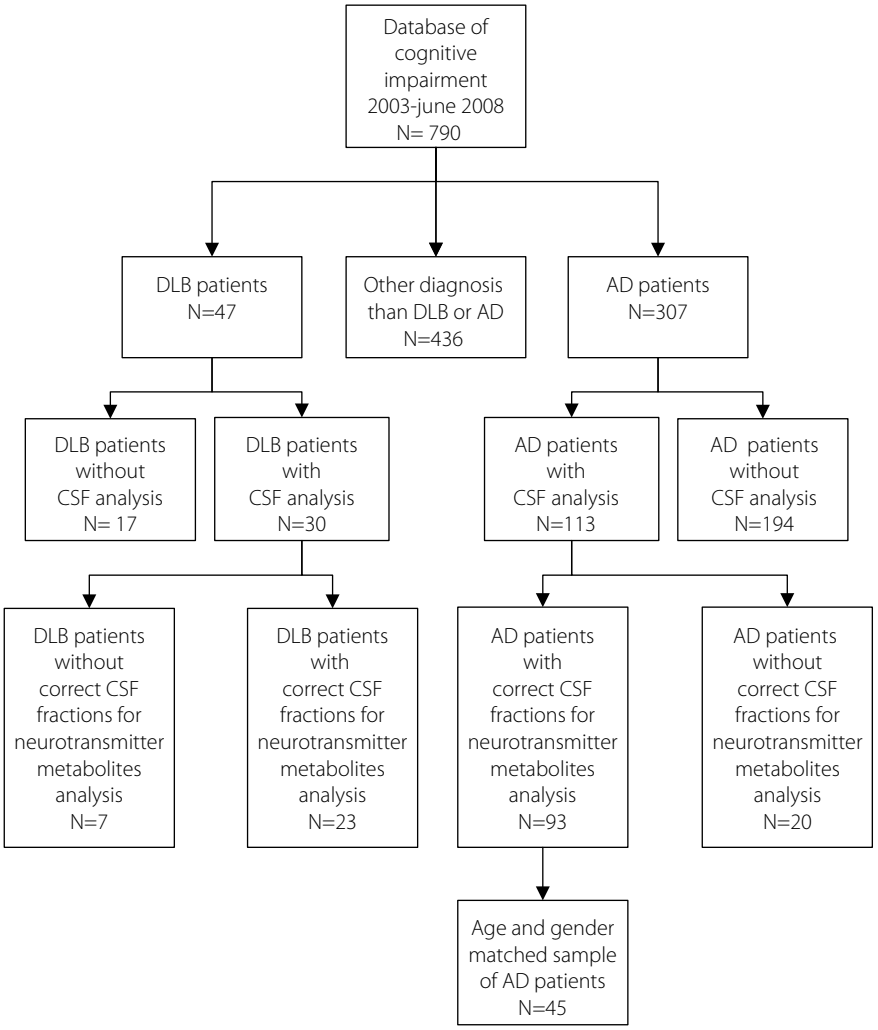

Figure 1 Flowchart of patient inclusion in this study.

AD: Alzheimer's disease, DLB: dementia with Lewy Bodies, CSF: cerebrospinal fluid, N: number. CSF was obtained during the initial diagnostic assessment upon presentation.

The concentration of MHPG in CSF is independent of the fraction, however, since the concentrations of the neurotransmitter metabolites HVA and 5-HIAA do depend on the CSF fraction, (Brautigam et al. 1999) only patients with separate collection of the 8th-10th (±2) milliliter fraction were included for analysis. Diagnostic evaluation included a detailed medical history, systematic physical and neurological examination, routine laboratory testing and a brain MRI-scan. Cognitive function was assessed using the Mini Mental State Examination (MMSE). (Folstein et al. 1983) Additionally, 15 DLB patients underwent neuropsychological assessment. Symptoms of parkinsonism were assessed using the Hoehn and Yahr score. Hetero-anamnesis was employed to assess the presence of visual hallucinations and fluctuations in cognition. The clinical diagnosis was established by either a specialized neurologist or geriatrician. Out of 93 eligible AD patients from the memory clinic database, an age and gender matched group of 45 AD patients was randomly drawn. The clinical diagnosis of these patients was established by a multidisciplinary team consisting of a geriatrician, a neurologist and a neuropsychologist.

In April 2010 a single rater (MBA) reassessed the final clinical diagnosis by clinical chart review in order to improve diagnostic certainty. Reassessment of the clinical diagnosis was performed after a follow-up period of 12 months or longer (Table 1) according to the international consensus criteria for DLB (McKeith et al. 2005) and AD. (McKhann et al. 1984) The use of medication (serotonergic as well as dopaminergic medication), the presence of white matter lesions and the presence of behavioral disorders, hallucinations and symptoms of autonomic dysfunction were recorded to enable subgroup analysis. The local Institutional Review Board has approved of this study which was conducted according to the Helsinki Declaration.

CSF parameters

CSF samples were collected in polypropylene tubes, centrifuged, aliquoted, and stored at -80°C until analysis. We aimed for separate collection of the 8th-10th ml (±2) fraction for analysis of neurotransmitter metabolites. The following CSF variables were taken into account for the present study: $A\beta_{42}$, t-tau, p-tau, MHPG, 5-HIAA and HVA; all parameters were analyzed within 4 weeks after CSF collection.

The methods of analysis of $A\beta_{42}$, t-tau, p-tau, MHPG, HVA and 5-HIAA in CSF, their validation and reference values have been previously described. (Abdo et al. 2004; Brautigam et al. 1999; de Jong et al. 2006)

Table 1 Characteristics of the diagnostic groups

Demographic characteristics	DLB	AD	p-value[b]
Number of patients	23	45	
Age, yrs[a]	71.6 (9.3)	71.6 (9.4)	NS (p=0.89)
Number of men (%)	18 (78)	34 (76)	NS (p=0.88)
Disease duration, months[a]	38.8 (26.4)	33.0 (28.1)	NS (p=0.42)
Cognitive function, MMSE[a]	23.0 (4.2)	19.5 (5.3)	<0.05
Duration of follow up, months	55.9 (30.3)	49.7 (32.4)	NS (p=0.75)
Vascular co-morbidity (%)[a]	5 (22)	12 (27)	NS (p=0.73)
Autonomic dysfunction (%)[a]	13 (76.5)	9 (20.5)	<0.001
Orthostatic hypotension (%)	2/ (11.8)	1 (2.3)	<0.001
Urogenital dysfunction (%)	11 (64.7)	8 (18.2)	<0.001
Hallucinations (%)[a]	13 (57)	1 (2)	<0.001
Use of SSRI (%)[a]	4 (17)	3 (7)	NS (p=0.15)
Use of L-dopa (%)[a]	6 (26)	0 (0)	<0.001
Severity of parkinsonism, H&Y score[a]	2.5 (2.0-2.5)	NA	NA
CSF fraction analyzed, ml (lower margin)[c]	8.0 (7.0-9.0)	7.3 (6.8-8.0)	NS (p=0.18)
CSF fraction analyzed, ml (upper margin)[c]	11.0 (10.0-11.5)	10.0 (9.0-10.5)	<0.05

Data represent mean and standard deviation (in case of Gaussian distribution), median and interquartile range (in case of non- Gaussian distribution) or number and percentage.

[a] At the time of lumbar puncture

[b] P value for differences using student's T-test. Gender distribution, the presence of vascular comorbidity, autonomic dysfunction, hallucinations and the use of medication were analyzed using χ^2 test.

[C] for neurotransmitter metabolite analysis

DLB: dementia with Lewy bodies; AD: Alzheimer's disease; MMSE: mini mental state examination; SSRI: selective serotonine reuptake inhibitor; L-dopa: levodopa; H&Y score: Hoehn and Yahr score; NA: not applicable; CSF: cerebrospinal fluid

Statistical Analysis

Between-groups analysis was performed using the Student's t-test in case of normally distributed data. Non-Gaussian distributed data were analyzed using the Mann-Whitney test. Pearson correlation coefficient was used to analyze correlations. Prior to correlation analysis, non-Gaussian distributed data were log-transformed to meet assumptions of normality and homogeneity. Logistic regression analysis was used to analyze relations between categorical variables.

Multivariate logistic regression and receiver operator characteristic (ROC) analysis were used to evaluate the diagnostic value of CSF parameters. Statistical analysis was carried out using GraphPad Prism version 4 (San Diego, CA) and SPSS software version 16.0 (Chicago, IL).

Results

Patients

At the time of the clinical chart review, 23 patients fulfilled the diagnostic criteria for DLB (6 possible DLB, 16 probable DLB, and 1 definite DLB) and 45 patients fulfilled the diagnostic criteria of AD (44 probable AD, and 1 definite AD). None of the patients complied with the criteria for mild cognitive impairment (MCI). (Petersen et al. 1999) Demographic characteristics are shown in Table 1. Six DLB patients and two AD patients had deceased at the time of the chart review.

The initial diagnoses, prior to CSF analysis were possible AD (n=18), probable AD (n=19), cognitive disorder not further specified (n=6), Creutzfeldt-Jakob disease (n=1) and possible DLB (n=1) for the AD patients; and for the DLB patients: possible DLB (n=10) , possible AD (n=3), Parkinson's disease (n=5), corticobasal degeneration (n=1), multiple system atrophy (n=1), cerebral small vessel disease (n=2) and psychogenic complaints (n=1).

Disease duration, gender and age at the time of lumbar puncture were similar in AD and DLB. MMSE score was lower in AD ($p<0.05$). Six DLB patients, and none of the AD patients, used dopaminergic therapy (median 375 mg; range 62.5-800 mg levodopa/day).

CSF parameters

The results of the CSF analysis are presented in Figure 2. The concentrations of 5-HIAA ($p<0.01$), HVA ($p<0.05$) and especially MHPG, t-tau and p-tau (all $p<0.0001$) were lower in DLB as compared to AD whereas the $A\beta_{42}$ concentrations tended to be higher in DLB than in AD ($p = 0.06$). We did not find an increase in median MHPG ($p=0.53$) or HVA concentrations ($p=0.43$) in DLB patients using dopaminergic medication relative to naïve DLB patients, hence these patients were not excluded from further data analysis.

Compared to our reference values, 72.7% of AD patients had a decreased CSF $A\beta_{42}$ (≤ 500 ng/l) as opposed to 45.5% of DLB patients ($p<0.05$). CSF t-tau was increased (≥ 350 ng/l) in 13.6% of DLB patients, compared to 84.1% of AD patients, whereas p-tau was increased (≥ 85 ng/l) in 0% of the DLB patients as opposed to 70.5% of the AD patients (all $p<0.0001$).

The above described differences in CSF parameters were present in AD and DLB patients irrespective of the certainty of diagnosis (i.e. possible or probable).

We demonstrated no association between the occurrence of behavioral problems, use of serotonergic medication, presence of white matter lesions on MRI or symptoms of autonomic dysfunction on one hand, and levels of the three neurotransmitter metabolites and brain-specific proteins t-tau, p-tau, or $A\beta_{42}$ on the other hand, neither in the entire group, nor in the DLB and AD patient groups separately. However, hallucinations were associated with lower MHPG (odds ratio (OR) 4.8, 95% CI 1.3-17.2 $p<0.001$) and HVA (OR 6.6, 95% CI 1.5-28.6, $p<0.001$) in DLB patients.

Repeating the analyses while adjusting for age, disease duration and cognitive function did not markedly change our results.

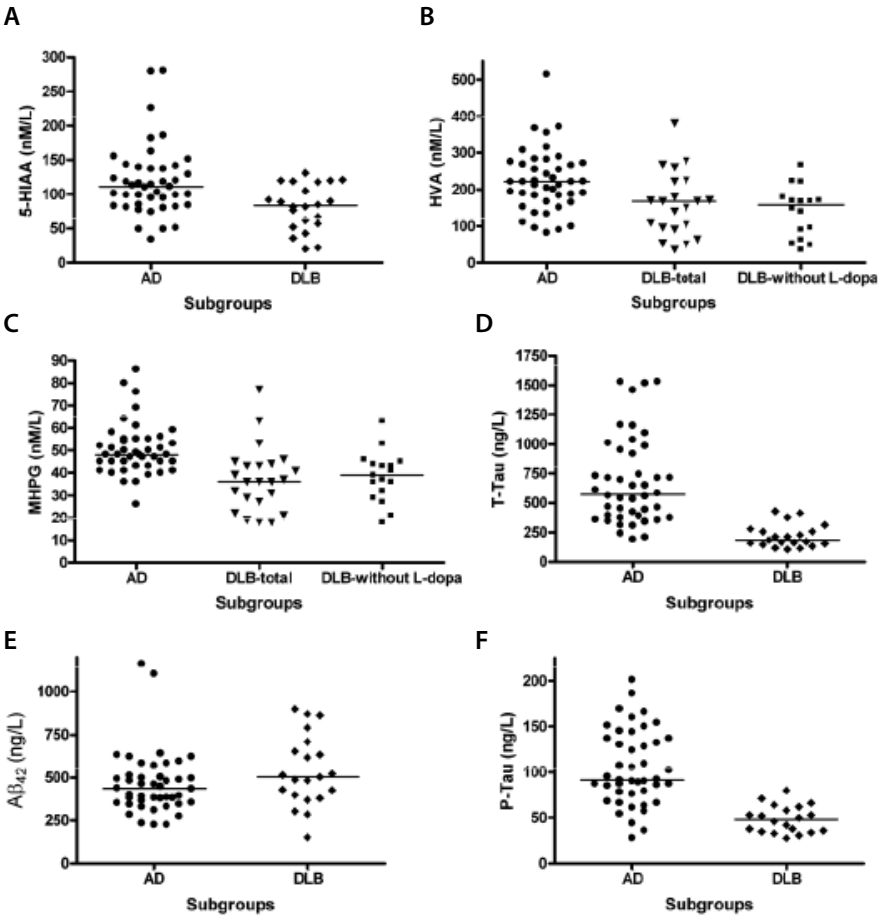

Figure 2 Scatterplots of the CSF concentrations of (**A**) 5-HIAA (nM), (**B**) HVA (nM), (**C**) MHPG (nM), (**D**) T-Tau (ng/L), (**E**) $A\beta_{42}$ (ng/L) and (**F**) P-Tau (ng/L) in DLB and AD subgroups.

Diagnostic accuracy

Univariate logistic regression analysis (carried out to discriminate DLB from AD) revealed that the sensitivity and specificity exceeded 80% for both t-tau and p-tau (Table 2). To assess the diagnostic value of neurotransmitter metabolites over the currently used combination of t-tau, p-tau and $A\beta_{42}$, we performed multivariate logistic regression analysis using block entry regression.

The first block consisted of the currently used brain specific proteins resulting in a Nagelkerke R^2 of 0.77 ($p<0.001$, model $\chi^2(3)=49.8$). In the second block MHPG, HVA and

Table 2 ROC-analysis of CSF parameters in DLB vs. AD

CSF variables	Number of patients[a]	Cut-off point	Sensitivity (%)	Specificity (%)	Area under the curve (95% CI)	Likelihood ratio[b]
Univariate analysis						
5-HIAA (nM)	DLB n=22, AD n=43	<92.5	72.1	68.2	0.72 (0.58-0.86)	2.10
MHPG (nM)	DLB n=22, AD n=43	<44.5	74.4	78.3	0.81 (0.68-0.92)	4.43
HVA (nM)	DLB n=22, AD n=43	<182.0	76.2	70.0	0.69 (0.54-0.85)	2.54
$A\beta_{42}$ (ng/L)	DLB n=21, AD n=44	>482.0	62.0	65.0	0.65 (0.49-0.81)	1.78
P-tau (ng/L)	DLB n=21, AD n=44	< 67.0	81.0	95.0	0.92 (0.86-0.99)	16.19
T-tau (ng/L)	DLB n=20, AD n=44	<294	90.4	90.0	0.95 (0.91-1.0)	9.05
Multivariate analysis						
Model 1[c]	DLB n=22, AD n=42	>0.42	92.9	90.0	0.96 (0.92-1.0)	9.29
Model 2[d]	DLB n=22, AD n=44	>-0.455	97.6	95.0	0.99 (0.97-1.0)	39.6

[a] Due to missing data points, not all CSF parameters were available in all patients.

[b] Likelihood ratio: sensitivity/(1-specificity)

[c] y= -5.098 + 0.005x p-tau, + 0.020x t-tau – 0.002x $A\beta_{42}$. AUC: 0.96 (0.92-1.0)

[d] y= -13.965 + 0.072x p-tau, + 0.021x t-tau + 0.143x MHPG – 0.006x $A\beta_{42}$. AUC: 0.99 (0.97-1,0)

ROC-: receiver operating characteristic; DLB: dementia with Lewy bodies; AD: Alzheimer's disease; CSF: cerebrospinal fluid; 5-HIAA: 5-hydroxyindolacetic acid; HVA: homovanillic acid; MHPG: 3-methoxy-4-hydroxyphenylethyleneglycol; $A\beta_{42}$: amyloid β42; p-tau: phospho-tau; t-tau: total tau protein; 95 CI: 95% confidence interval

5-HIAA were added. Only MHPG showed significant added value to the constructed model based on block 1, improving Nagelkerke R^2 from 0.77 to 0.87 ($p<0.001$, model $\chi^2(4)=59.4$). ROC-curve analysis of the first model (based on t-tau, p-tau and $A\beta_{42}$ demonstrated a sensitivity of 92.9% and a specificity of 90% (AUC 0.96). The subsequent addition of MHPG resulted in further improvement of the diagnostic accuracy with a sensitivity of 97.6% and a specificity of 95% (AUC 0.99).

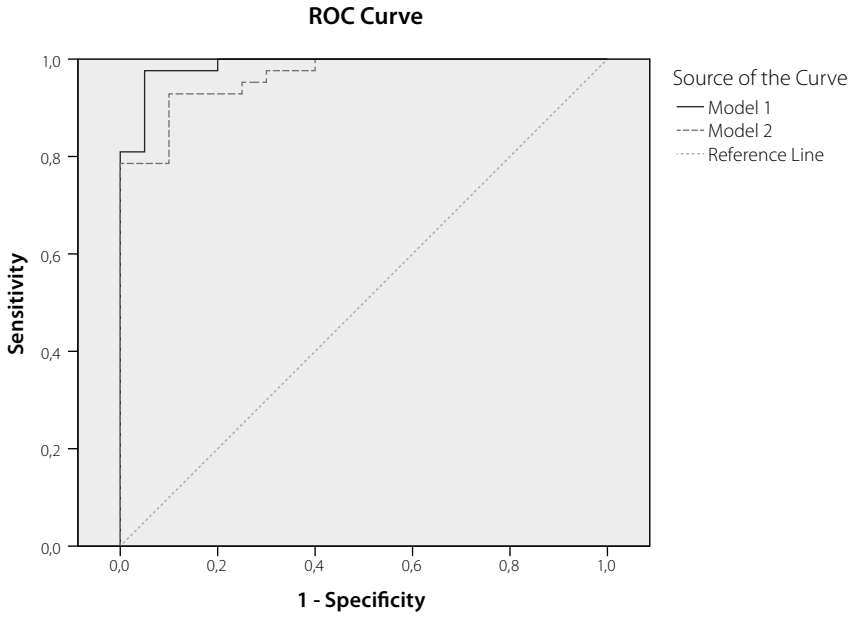

Figure 3 ROC curves of the models 1 and 2.

Model 1: y= -13.965 + 0.072x p-tau, + 0.021x t-tau + 0.143x MHPG – 0.006x $A\beta_{42}$. AUC: 0.99 (0.97-1.0)
Model 2: y= -5.098 + 0.005x p-tau, + 0.020x t-tau – 0.002x $A\beta_{42}$. AUC: 0.96 (0.92-1.0)

Discussion

We found that CSF concentrations of 5-HIAA, HVA, MHPG, t-tau and p-tau were significantly lower in DLB than in AD, whereas CSF $A\beta_{42}$ tended to be higher in DLB. Most important, the combination of MHPG, p-tau, t-tau and $A\beta_{42}$ analysis discriminated between AD and DLB with high diagnostic accuracy.

We are the first to rigorously investigate CSF MHPG in a large group of DLB patients. Only one earlier study examined CSF MHPG concentrations in DLB and, although the sample size was small (n = 8 subjects) findings are consistent with ours. (Weiner et al. 1996) Moreover, our findings of lower concentrations of CSF MHPG are consistent with previous neuropathological studies, demonstrating degeneration of the locus coeruleus (Mann et al. 1980) and decreased concentrations of norepinephrine in putamen and neocortex, as compared to controls and AD patients. (Ohara and Kondo 1998) Moreover, these findings are also compatible with the neuropathologically observed degeneration of nigrostriatal dopaminergic neurons in DLB, (Dickson et al. 2009) because reduced availability of dopamine results in a decrease of dopamine-derived neurotransmitter metabolites.

Interestingly, even though 5-HIAA and HVA were significantly lower in DLB as compared to AD, both did not contribute significantly to the constructed model, possibly because the direction of the effects were similar to MHPG and and the strong correlation between CSF HVA and MHPG r 0.618, $p< 0.001$.

The brain specific proteins t-tau, p-tau and $A\beta_{42}$ have been studied abundantly in both AD and DLB. We confirmed previous results that CSF $A\beta_{42}$ concentrations are low in DLB and close to the levels observed in AD patients, (Andreasen et al. 2001; Bibl et al. 2006; Clark et al. 2003; Gomez-Tortosa et al. 2003; Kanemaru et al. 2000; Tschampa et al. 2001; Verbeek et al. 2003) possibly reflecting the AD-like pathology observed in a proportion of the DLB patients upon post-mortem examination, substantially limiting its discriminating properties. We also found increased t-tau levels in AD, but generally normal levels in DLB. Previous studies demonstrated conflicting results; some studies found elevated t-tau concentrations in DLB patients, (Andreasen et al. 2001; Kanemaru et al. 2000) whereas other studies showed normal t-tau concentrations, (Clark et al. 2003; Kasuga et al. 2010; Tschampa et al. 2001) as we did. In our study, as in two other reports (Kasuga et al. 2010; Parnetti et al. 2001) p-tau concentrations were normal in DLB, although conflicting results were also reported. (Buerger et al. 2002) These discrepancies underscore the importance of additional CSF parameters, in order to compose a more comprehensive and robust CSF profile.

Our results imply that the addition of CSF MHPG to the currently used analysis of brain-specific proteins can further improve the diagnostic differentiation between DLB and AD. The recent observation that up to 18% of patients suffering from dementia are treated with neuroleptics (Guthrie et al. 2010) further stresses the importance of diagnostic accuracy in early disease stages, because the use of neuroleptics in DLB patients is contra-indicated for fear of increased physical and cognitive deterioration, as well as increased mortality. (Aarsland et al. 2005)

Cognitive dysfunction is often assessed using the MMSE score. This score, however, has certain disadvantages, as it is an inaccurate method of cognitive assessment. For example, it is known that, as cognition is known to fluctuate in DLB, test – re-test variability is substantial. However, the MMSE is easy to perform and still the most commonly used

cognitive screening assessment in daily practice, and therefore used in this study as well. The slightly lower MMSE scores in the AD subgroup might reflect the often more pronounced amnestic and orientation problems observed in AD.

This difference in MMSE likely does not affect the results for CSF t-tau, p-tau and $A\beta_{42}$, since it is known that CSF biomarker levels are hardly dependent on disease state and severity, especially in AD. It is, however, unknown whether this also applies to DLB and to CSF neurotransmitter metabolite levels. Therefore, to acknowledge these differences in MMSE score, but also in age and sex, between AD and DLB we included these parameters in our models, but this modification did not result in an altered AUC.

This study has several potential drawbacks. First, the retrospective design may have introduced selection bias. Only DLB and AD patients who underwent a lumbar puncture as part of their diagnostic workup were included in this study, possibly leading to selection of more atypical phenotypes. However, we included a substantial number of probable and even definite DLB patients, who showed similar CSF patterns. Second, the clinical diagnosis was not confirmed neuropathologically in most patients. Misclassification may therefore have occurred. However, accuracy of the final clinical diagnosis was optimized using the following approach: thorough clinical and ancillary investigations at baseline; extensive follow-up (53 months for DLB and 50 months for AD patients) to monitor disease progression and development of new diagnostic signs; and establishing the diagnosis according to international consensus criteria in a specialized clinic. Third, the proposed model based on CSF biomarkers warrants validation in an independent and larger cohort. Despite these drawbacks, our results underline the importance of CSF analysis for the differentiation between dementia syndromes, specifically between AD and DLB. Moreover the addition of CSF MHPG to the currently used analysis of the brain-specific proteins t-tau, p-tau and $A\beta_{42}$ may further improve this diagnostic differentiation. These results warrant validation in a prospective study with preferably neuropathological confirmation of the diagnosis and inclusion of patients with other types of dementia.

4 A prospective study to evaluate the diagnostic approach in parkinsonism

4.1 A prospective study to evaluate the diagnostic approach in parkinsonism

Design of the study

Based on

MB Aerts, RAJ Esselink, WF Abdo, FJA Meijer, G Drost, N Norgren, M Janssen, GF Borm, BR Bloem, MM Verbeek, Ancillary investigations to diagnose parkinsonism; a prospective clinical study, *submitted*.

Summary

In everyday practice, differentiating between Parkinson's disease (PD) and atypical parkinsonism (AP) is challenging due to overlap in clinical presentation. Various ancillary investigations are available to facilitate the differential diagnosis, but have not yet been compared within one study. We will conduct a prospective study to investigate the individual and relative value of electromyography (EMG) of the anal sphincter, analysis of cerebrospinal fluid (CSF), magnetic resonance imaging of the brain (MRI) and 123I-iodo-benzamide Single Photon-Emission Computed Tomography (IBZM-SPECT) for discriminating between PD and AP. Our aim is to develop a diagnostic model based on baseline clinical characteristics, and ancillary investigations.

Consecutive patients with parkinsonism referred to our outpatient department are invited to participate. Baseline assessment includes extensive neurological examination, MRI, anal sphincter EMG, IBZM-SPECT and lumbar puncture. The clinical diagnosis after 3-year follow-up will serve as the silver standard diagnosis, based on additional neurological signs, rate of progression and treatment response. Two movement disorder specialists will establish this silver standard diagnosis in consensus. Univariate and multivariate analysis will be performed to determine which of the baseline assessments best predicts the silver standard diagnosis.

This study has the potential to clarify the individual and relative diagnostic value of MRI, EMG, IBZM-SPECT and CSF analysis for discriminating between PD and AP.

Introduction

Differentiation between Parkinson's Disease (PD) and the various forms of atypical parkinsonism (AP) such as multiple system atrophy (MSA), progressive supranuclear palsy (PSP), and corticobasal syndrome (CBS) can be difficult due to overlap in clinical symptoms, especially early in the course of the disease. Although the clinical diagnosis after several years of follow-up highly correlates with the neuropathological diagnosis upon post-mortem examination, the diagnostic accuracy of the initial diagnosis varies greatly and can be as low as 30%. (Hughes et al. 2002; Litvan et al. 1996; Litvan et al. 1997) While difficult, a correct diagnosis is important for adequate treatment and patient counseling, as well as for research purposes.

Various ancillary investigations are available to facilitate the differential diagnosis, but different modalities of ancillary investigations have not yet been compared in one study. We sought to investigate the diagnostic accuracy of four different tests, which have all been proposed as a diagnostic tool for the neurologist in daily practice. These tests include brain magnetic resonance imaging (MRI), electromyography (EMG) of the anal sphincter, analysis of the cerebrospinal fluid (CSF) and 123I-iodobenzamide Single Photon-Emission Computed Tomography (IBZM-SPECT).

MRI characteristically does not show abnormalities in PD, whereas specific signs mark AP, such as the hummingbird sign (midbrain atrophy) for PSP, the putaminal rim (putaminal atrophy) and hot cross bun sign (atrophy of pontine fibres) for MSA, and the asymmetrical cortical atrophy in CBS. (Schrag et al. 2000) A recently published study describes the pilot data of this study. (Meijer et al. 2012)

EMG of the anal sphincter can detect denervation of the external anal sphincter due to degeneration of Onuf's nucleus in up to 70% of PSP and MSA patients. (Papp and Lantos 1994; Scaravilli et al. 2000) Such abnormalities are only rarely observed in PD patients in the early phase of the disease, (Podnar and Fowler 2004; Vodusek 2005; Winge et al. 2010) but can be present later in the course of the disease. (Paviour et al. 2005)

CSF analysis of brain specific proteins and neurotransmitter metabolites in PD patients are generally within normal range, whereas in especially MSA a characteristic profile with elevated brain specific proteins, especially tau protein and neurofilaments, is seen. (Abdo et al. 2007; Aerts et al. 2011b) The observed abnormalities in CSF possibly reflect the increased neuronal damage as observed in AP, as elevated concentrations of especially the brain-specific proteins can be observed in other quickly progressive neurodegenerative diseases like Creutzfeldt-Jakob disease. (van Eijk et al. 2010)

IBZM-SPECT enables imaging of the post-synaptic dopamine D2 receptors. PD patients generally show up-regulation of these D2 receptors in the striatum, whereas both MSA and PSP patients show decreased density of dopamine D2 receptors in the striatum. (Churchyard et al. 1993; Kim et al. 2002; Plotkin et al. 2005) However, sensitivity can be limited due to overlapping ranges between PD and AP. (Seppi et al. 2004; Verstappen et al. 2007)

The studies cited above demonstrate the potential merits of the various investigations when used in isolation. However, it is more difficult to compare different sorts of ancillary investigations in their ability to discriminate between PD and AP. Consequently, it remains uncertain which ancillary investigation(s) to choose first when the clinician is uncertain about the underlying etiology of a patient with parkinsonism. We therefore have designed a prospective clinical trial to investigate the individual and relative value of three types of ancillary investigations in discriminating PD and AP. (Figure 1)

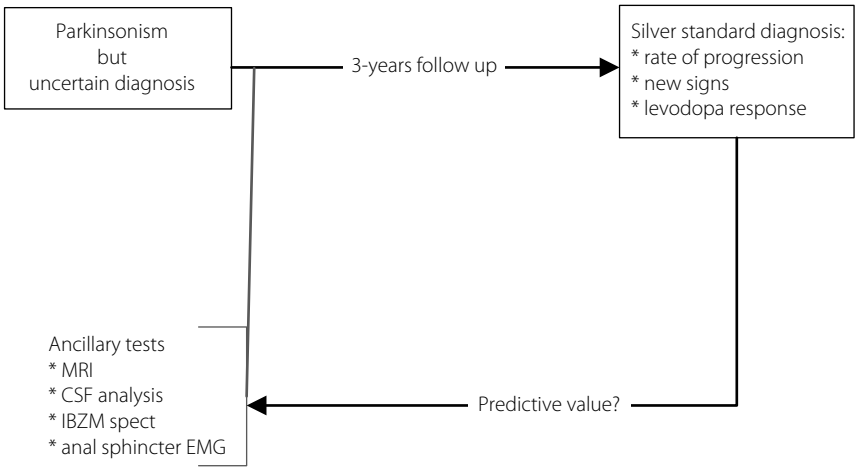

Figure 1 The study design.

At baseline several ancillary tests are performed. After follow up a silver standard diagnosis is performed. Looking back, the predictive value of the ancillary tests at baseline is analyzed. MRI: magnetic resonance imaging; CSF: cerebrospinal fluid; IBZM-SPECT: 123I-iodobenzamide Single Photon-Emission Computed Tomography; EMG: electromyography

Methods

Design

Prospective, longitudinal study design

Participants and setting

Consecutive new patients with a hypokinetic-rigid syndrome, referred between September 2003 and November 2006 to the movement disorders clinic of the Department of Neurology at the Radboud University Nijmegen Medical Centre, were invited to participate. Inclusion and exclusion criteria are provided in Table 1. The clinical examinations and ancillary investigations are performed in this hospital.

Medical ethical approval is obtained by the local Institutional Review Board (2002). All patients have to sign the informed consent form after detailed explanation of the procedures.

Table 1 Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
- Hypokinetic rigid syndrome of neurodegenerative origin - Aged >18 years	- Instable comorbidity - Patient unfit to consent - Thrombopenia - A medical history of brain surgery

Methods

After informed consent and within 6 weeks of the initial visit, all patients undergo a structured interview, detailed and standardized neurological examination, magnetic resonance imaging (MRI) scan, a lumbar puncture, IBZM-SPECT and electromyography (EMG) of the anal sphincter. After three years all patients will be re-examined by the initial investigator. The final clinical diagnosis will be established in consensus and according to the clinical criteria by neurologists specialized in movement disorders. These neurologists are blinded for the results of the ancillary investigations, except MRI because this is now nearly routinely used in clinical practice. Previously published data show a very high concordance between the neuropathological diagnosis and the clinical diagnosis after at least 2 years follow up by a movement disorder specialist (PPV 99%). (Hughes et al. 2002) Hence, this final clinical diagnosis serves as a surrogate 'silver standard' to calculate the diagnostic accuracy of CSF analysis, IBZM-SPECT and anal sphincter EMG to differentiate between PD and AP. (Flowchart 1)

Interview and neurological examination

Interview and neurological examination are performed by two independent physicians, not directly involved in patient care (WFA, RAJE). Using a structured interview the following items are assessed: medical history, used medication, presenting complaints and progression of the disease, most affected body site, balance and fear of falling, activities in daily living, and quality of sleep. In addition, the following clinimetric scales are scored: Unified Parkinson's Disease rating scale (UPDRS) III and IV and Hoehn and Yahr score, (Hoehn and Yahr 2001) International Cooperative Ataxia Rating Scale (ICARS), (Trouillas et al. 1997) and Mini mental state examination (MMSE), (Folstein et al. 1983) for cognitive assessment. Definitions can be found in Table 2.

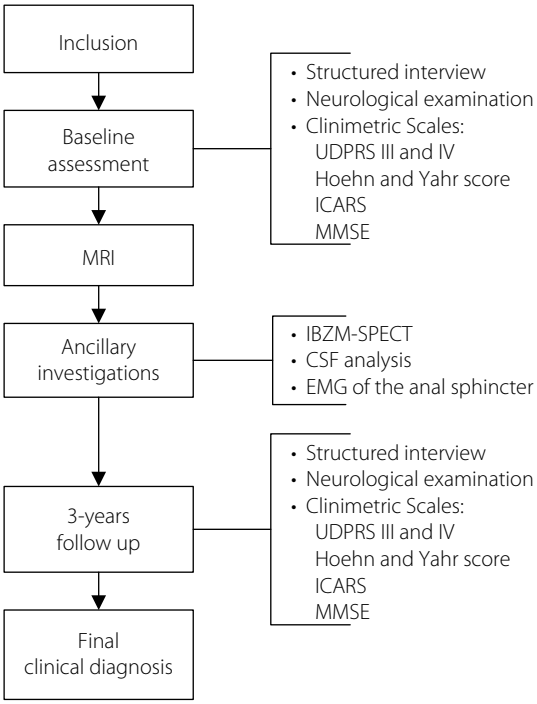

Figure 2 Flowchart study design.

MRI: magnetic resonance imaging; UPDRS: unified Parkinson's Disease rating scale; ICARS: International Cooperative Ataxia Rating Scale; MMSE: mini mental state examination; battery; IBZM-SPECT: 123I-iodobenzamide Single Photon-Emission Computed Tomography; CSF: cerebrospinal fluids; EMG: electromyography

MRI

All patients receive a brain MRI at first presentation performed on a 1 or 1.5 Tesla MRI scanner. The scanning protocol is not standardized as the studies are made in the clinical setting, and not for research purposes. All MRI scans will be evaluated by an experienced neuroradiologist (FJAM) blinded for clinical symptoms and outcome in a systematic fashion. To assess interrater variability, a second neuroradiologist, also blinded for clinical symptoms and outcome, will evaluate a consecutive subset of MRI studies. The presence of the following abnormalities will be noted: putaminal T2 hypo- and hyperintensity changes, putaminal rim sign, putaminal atrophy, frontal lobe and parietal lobe atrophy, lateral, third and fourth ventricle dilatation, midbrain and pontine atrophy, hummingbird sign, atrophy of the cerebellum and cerebellar vermis, atrophy of the medulla oblongata, pontine T2 hyperintensity and hot cross bun sign, dilated perivascular spaces, lacunar infarctions and white matter changes. White matter changes are scored according to the

age related white matter changes criteria**.** (Wahlund et al. 2001) For validation purpose the scoring system proposed by Yekhlef (Yekhlef et al. 2003) combining several MRI abnormalities in cortical, putaminal, midbrain and pontocerebellar regions will be used. In accordance with these criteria, a cut-off value of 8 is set to discriminate PD from AP. Analysis of these data is published recently. (Meijer et al. 2012)

Table 2 Definitions of the clinical parameters

History	All indicators are derived from the history taking. Hence, these are questions asked to the patient, not observations by the examiner.
Age	Continuous parameter
Course of the disease	Either stable, slowly progressive, or quickly progressive
Use of walking aids	Walking with a stick, walker or wheelchair
Cognitive dysfunction	Whether the patient felt that cognition had declined more than compared to people the same age (yes or no)
Presence of falls	Whether the patient falls or has fallen the past year (yes or no)
Presence of night time stridor	Whether the patient has inspiratory stridor at night time (yes or no)
Hypersalivation	Does the patient experience night hypersalivation (yes or no)
Ability to cycle	Is de patient still able to cycle despite the disease (yes or no)
Autonomic dysfunction	Does the patient experience urgency, urge incontinence, erectile dysfunction or lightheadedness upon standing (yes or no)
Independence of care	Is the patient fully independent in daily self care (yes or no)
Dysphagia	Does the patient have trouble swallowing (yes or no)
Neurological examination	All indicators are derived from the neurological examination. Hence, these are observations by a trained examiner
Romberg	The patient is asked to stay with the eyes closed and feet together. Romberg's test is disturbed when balances is lost when closing the eyes. (yes or no)
Pathological reflexes	Two or more of the following reflexes: glabellar, snout gasp, palmomental, masseter reflex
Cognitive assessment MMSE Fluency	Cognitive assessment (Folstein et al. 1983) (range 0-30) The average of two phonetical (the patient is asked to name as many words with both S and T as starting vowel) and one categorical trial (naming as many animals as possible). Each trial lasts 60 seconds.

Table 2 Continued

Ataxia	Score of separate items, see ICARS reference below
Dysarthria	Presence of slurred speech,
Finger- nose test	Patient is asked to point the finger to the nose; assessed for dysmetria tremor.
Heel shin test	Patient is asked to bring the heel to the other legs shin and slide down; assessed for dysmetria and tremor
Tandem gait	The patient has to walk 10 consecutive staps in tandem, not one single side step is allowed. (range 0 (without side steps) – 4 (not able to perform)
ICARS total score	International Cooperative Ataxia Rating Scale (Trouillas et al. 1997)
UPDRS axial score	Composite score of all axial parameters tested in the UPDRS including speech and face assessment, axial rigidity, rising from a chair, posture, gait and assessment of postural stability
Disease stage	Disease stage: 0: normal, 1: disturbed gait but walking independently; 1.5: disturbed gait, intermittent use of walking aids; 2: disturbed gait, permanent use of walking aids; 2,5: disturbed gait, intermittent use of wheelchair; 3: disturbed gait, permanent use of wheelchair; 4: death.
Schwab and England score	Disease severity (Fahn and Elton 1987)
Myoclonus	Brief, shock-like movements (yes or no)
Autonomic dysfunction	
Early orthostatic hypotension	A drop of 30 mmHg in systolic or 15 mmHg in diastolic blood pressure directly after standing
Late orthostatic hypotension	Similar after 3 minutes of standing
Eye movements	
Saccadic intrusions	The disturbance of smooth pursuit by saccadic intrusions (yes or no)
Slow saccades	Slowed velocity of saccadic eye movements (yes or no)
Multistep saccades	The saccadic eye movements cannot be performed in one attempt, multiple shorts saccadic eye movements are needed (yes or no)
Supra nuclear palsy	Limited range of vertical eye movements (yes or no)

CSF

CSF samples are collected in polypropylene tubes, centrifuged, aliquoted, and stored at -80°C until analysis. We aim for separate collection of the 9th-11th ml fraction for analysis of neurotransmitter metabolites. All CSF analysis is performed by laboratory technicians blinded for clinical symptoms and outcome. The following CSF variables are analyzed: Amyloid β_{42} ($A\beta_{42}$), total tau protein (t-tau), tau protein phosphorylated at Thr181 (p-tau), α-synuclein, neuronspecific enolase (NSE), S-100B, Glial fibrillary acidic protein (GFAP), myelin basic protein (MBP), neurofilament light chain (NFL), neurofilament heavy chain (NFH), L-dopa, 3-Methoxy tyrosine (3MT), 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG), 5-hydroxyindolacetic acid (5-HIAA), homovanillic acid (HVA), lactate, total protein, blood pigments and the total erythrocyte count; The erythrocyte count and blood pigments are analyzed within 2 hours after CSF collection, all other parameters are analyzed within 4 weeks after CSF collection.

$A\beta_{42}$ concentrations in CSF are analyzed using the Innotest amyloid β42 assay (Innogenetics, Ghent, Belgium; linearity up to 2000ng/L, interassay variation coefficient (ICV) 6.4%). T-tau concentrations in CSF are analyzed using the Innotest hTau assay (Innogenetics, Ghent, Belgium; linearity up to 1200ng/L, ICV 8.3%). P-tau concentrations in CSF are analyzed using the Innotest Phospho-$Tau_{(181)}$ assay (Innogenetics, Ghent, Belgium; linearity up to 500ng/L, ICV 3.8%). Both NSE and S-100B concentrations in CSF are analyzed in an immunoluminometric assay (Byk Sangtec, Dietzenbach, Germany) by using the Liaison automated analyzer (Byk, Sangtec). The assays are linear up to 100 µg/L (NSE) and 30 µg/L (S-100B). CSF GFAP is analyzed by using a homemade sandwich ELISA (linear up to 250 µg/L; ICV <14%). (van Geel et al. 2002) MBP is analyzed using a commercial ELISA (DSL, Webster, Texas; linearity up to 10 µg/L, ICV <10%. NFH is measured according to previously described methods (Abdo et al. 2007; Van Geel et al. 2005) ICV <18% for both NFH and NFL), NFL is measured by a commercial 2-site solid phase sandwich ELISA (UmanDiagnostics, Umea, Sweden; Detection limit: 31ng/L linearity up to 10 µg/L, ICV <6 %, L-dopa and 3MT are analyzed using HPLC with fluorimetric detection; excitation 278nm and emission at 325nm (linear up to 160 nM; ICV <7%). MHPG, HVA and 5-HIAA in CSF are measured according to previously described methods. (Abdo et al. 2004; Brautigam et al. 2002) Because the concentrations of HVA and 5-HIAA vary in the different fractions of CSF, (Brautigam et al. 2002) the 9th-11th milliliter CSF fraction is used for analysis. The assays are linear within the following ranges: HVA, 0 to 4 µM; 5-HIAA, 0 to 2 µM; MHPG, 0 to 125 nM. ICV is ≤4.8% in all three assays. The method of α-synuclein analysis and its validation is published previously. The linearity of the used assay ranges from 6 to 300ng/ml. The ICV is 3.5% at a concentration of 49ng/ml. The number of erythrocytes is manually counted in a counting chamber (volume 3 µl), blood pigments are analyzed using spectrophotometric analysis of centrifuged CSF (Perkin-Elmer, Groningen; The Netherlands) and calculation of the second derivative of the signal (which is linear with the concentration) in the spectrum between 573 and 578 nm is performed to quantify hemoglobin and the spectrum

between 460 and 478 nm is used for quantification of bilirubin. Lactate concentration is determined by the enzymatic conversion of lactate into pyruvaat and hydrogen peroxide in the presence of lactate oxidase and the subsequent conversion of hydrogen peroxide with 4-aminoantipyrine and N-ethyl-N-sulfopropyl-m-anisidine to quinoneimine in the presence of peroxidase, measured at 550nm. Total protein concentration is determined with the Lowry reaction and absorption measured at 720 nm. Both lactate and total protein are analyzed with an automated analyzer (Mira Plus; ABX, Eindhoven, The Netherlands).
For a comprehensive analysis of the various CSF parameters, we developed a scoring system. Only the best documented CSF parameters, based on own experience and literature, we have added in this model. Other parameters (e.g. cell count) are used to exclude other causes of disease, or are aimed for cross-sectional – post hoc analysis (e.g. α-synuclein). Moreover, we sought to weigh slightly abnormal values less heavily than significantly abnormal values. (Table 3) The individual cut-off values are assessed based on prior studies as well as the currently used cut-off values in our laboratory.

Table 3 CSF analysis paradigm

CSF parameter	Individual cut-off value	Score
T-tau	> 225 > 350	1 2
A β_{42}	< 500 < 350	1 2
NFL	> 2500 > 3000	1 2
MHPG	< 43 < 38	1 2
HVA And 5-HIAA	< 70 And < 100	1
Total score		9

T-Tau: total Tau ; A β_{42} : Amyloid β 42; NFL: neurofilament light chain ; MHPG: 3-methoxy-4-hydroxyphenyl-ethyleneglycol; HVA: Homovanillic acid; 5-HIAA: 5-hydroxyindolacetic acid

The following CSF parameters are incorporated in the model: T-tau, probably the best documented CSF parameter to discriminate PD and AP, with a first cut-off of >225ng/l (slightly elevated) (Abdo et al. 2007) and >350 ng/l as a second cut-off (significantly elevated); NFL, with a first cut-off of >2500ng/l (based on prior analyses (Abdo et al. 2007)) and >3000 ng/l as a second cut-off; Aβ42, with a first cut-off of <500ng/l and <350 ng/l as a second cut-off; (Aerts et al. 2011c) MHPG with a first cut-off of <43ng/l and <38ng/l as a

second cut-off; (Aerts et al. 2011c) and lastly the combination of HVA (<70mmol/l) and 5-HIAA (<100mmol/l). (Aerts et al. 2011c) In total, 9 points can be obtained. The cut-off for the discrimination between PD and AP is determined at 3 points, with a score below the cut-off leading to a CSF-based diagnosis of PD.

IBZM-SPECT

Cerebral SPECT imaging is performed 90 minutes after slow (30s) intravenous injection of 185 MBq 3-[123]iodo-6-methoxybenzamide, (iodobenzamide or IBZM, GE Healthcare, Eindhoven, the Netherlands) with a dual head gamma camera. The first 34 scans are made on a Siemens MultiSpect camera connected to an ICON computer (Siemens AG, Erlangen, Germany), the remaining on a Siemens ECAM (Siemens AG, Erlangen, Germany) connected to a HERMES work station (HERMES, Nuclear Diagnostics, Stockholm, Sweden). The cameras are especially calibrated for quantification using a traveling phantom and fitted with low-energy high-resolution collimators using a 15% energy window centered on the 159 keV photon energy-peak of iodine-123. Both heads perform a 180 degree circular motion and collected 64 projections (40 seconds per view) in a step-and-shoot mode using a 128 x 128 matrix with a zoom factor of 1.23. During scanning the patient's head is positioned in a head holder and the patient's head and shoulders are fixated to minimize movement during the scan. The radius of the detector orbit is kept as small as possible, usually 11-15cm.

Transaxial images will be reconstructed using filtered back projection with post-reconstruction filtering (Butterworth 8th order, cutoff 0.6) without attenuation or scatter correction. The head is reoriented to the canthus-meatus plane. The three consecutive slices with the highest striatal uptake (total thickness 14.6 mm) are selected for quantitative analysis. Fixed size regions of interest (derived from an anatomical brain atlas) are bilaterally drawn over the striatum and the occipital cortex enabling the calculation of striato-occipital ratio's. (van Royen et al. 1993) All drugs known to interfere with scanning will be withdrawn prior to scanning. (Table 4) Dopamine agonists and levodopa have to be discontinued for at least one week and >12 hours respectively. Quantitative and qualitative analysis of the IBZM-SPECT scans are performed by an independent nuclear medicine physician, blinded for clinical symptoms and outcome.

EMG of the anal sphincter

Standard needle EMG procedure will be performed. (Podnar and Fowler 2004) Subjects lay on their right side, with knees and hips flexed. A 37 mm long standard disposable concentric EMG needle with a diameter of 0.46 mm and a recording area of 0.07 mm^2 will be used. (Medelec Elite disposable concentric needles, Viasys Healthcare, Carefusion, San Diego, California, U.S.A.). The needle is placed into the subcutaneous external anal sphincter (EAS) muscles. On each side, one skin penetration and EMG analysis of at least two different positions in the EAS muscle will be performed.

Table 4 Medication interfering with IBZM-SPECT scanning

Amfetamine	Penfluridol (Semap)
Alizapride (Litican)	Pergolide (Permax)
Apomorfine (Apo-go)	Pimozide (Orap)
Benperidol (Frenactil)	Pipamperone (Dipiperon)
Bromocriptine (Parlodel)	Pramipexol (Sifrol)
Cabergoline (Dostinex)	Prochloorperazine (Stemetil)
Chloorpromazine (Largactil)	Promazine (Sparine)
Cinnarizine	Promethazine (Phenergan)
Clozapine (Leponex)	Perphenazine (Decentan)
Cocaine	Quetiapine
Flufenazine (Anatensol)	Quinagolide (Norprolac)
Flunarizine (Sibelium)	Raclopride
Flupentixol (Fluanxol)	Risperidone (Risperdal)
Haloperidol (Haldol)	Ropinirol (Requip)
Lisuride (Dopergin)	Rotigotine (Neupro)
Methylfenidaat (Ritalin)	Sulpiride (Dogmatil)
Metoclopramide (Primperan)	Zuclopentixol (Cisordinol)
Olanzapine (Zyprexa)	

The EMG activity of the EAS muscles is assessed in rest, during relaxiation and contraction. The EMG signals are amplified and filtered between 20Hz and 3 kHz and stored using the liveplay feature of the Medelec Synergy EMG equipment. (Synergy, Viasys Healthcare, Carefusion, San Diego, California, U.S.A.; software version 14, Oxford Instruments Medical, UK).

Visual inspection of these needle EMG investigations is performed at different gains by an independent clinical neurophysiologist (GD), blinded for clinical symptoms and outcome. A sensitivity of 20 µV/div with an acquisition duration of 100ms at rest, 100 µV/div with an acquisition duration of 100ms during moderate contraction, and a sensitivity of 200 µV/div with an acquisition duration of 1s during relaxation and full contraction are used.

Follow up

Three years after the inclusion visit, patients will be again seen on the outpatient clinic for a repeated structured interview and neurological examination by an independent physician, (WFA) blinded for test results and clinical records of the treating neurologist.

Clinical diagnosis

The clinical diagnosis is established in a systematic fashion by two movement disorder specialists, blinded for test results. All patient data are anonimized and sequentially presented to this panel: 1) clinical data and clinimetrics (UPDRS, MMSE, ICARS) upon inclusion, 2) disclosure of MRI results, 3) description of the reaction to dopaminergic medication, 4) disclosure of the clinical data and clinimetrics (UPDRS, MMSE, ICARS) after 3 years follow-up, and 5) disclosure of ancillary investigations. Each time the panel -in consensus- establishes a diagnosis (either PD or AP (not otherwise specified) and the corresponding degree of uncertainty (on a 0-100% rating scale), followed by a more specific diagnosis (e.g. IPD, MSA or PSP) always according to the international clinical criteria. (UK Parkinson's Disease Society Brain Bank clinical diagnostic criteria for PD, (Hughes et al. 1992) NINDS-SPSP criteria for PSP, (Litvan et al. 1996) Boeve criteria for CBS, (Boeve et al. 2003) McKeith Criteria for DLB, (McKeith et al. 2005) Gilman criteria for MSA, (Gilman et al. 2008) and Zijlmans criteria for VaP. (Zijlmans et al. 2004) The diagnosis prior to disclosure of the ancillary investigations (as defined above under (4)) is used as the silver standard to compare the ancillary investigations with.

Statistical analysis

Research questions

The objective of the study is to evaluate the added value of ancillary investigations to the clinical evaluation in the discrimination of PD and AP. Research questions include:

1. Evaluation of the initial clinical diagnosis as compared to the final diagnosis
2. The development of a diagnostic model based on baseline characteristics and clinical variables
3. The evaluation of the contribution of MRI scan variables, IBZM, CSF and EMG to the diagnostic accuracy in discriminating between PD and AP
4. The development of a diagnostic model based on baseline characteristics, clinical variables and ancillary investigations

In this article, we describe the overall design of the study including detailed methods, present baseline characteristics and the statistical methods for the proposed research questions.

Outcome measures

The primary outcome measure is the number of correct diagnoses (i.e. PD or AP) at the first diagnosis. Secondary outcome measures will include the number of correct specific AP diagnoses.

Analysis plan

We will analyze the diagnostic power of potential identifiers of atypical parkinsonism, obtained from history and neurological assessment, both for each identifier separately and for combinations of identifiers. Missing data points are imputed as normal. The discriminative power of an identifier or a combination of predictors is quantified using the area under the receiver operating curve (AUC).

Logistic regression with stepwise model selection is used to identify combinations of parameters that had optimal diagnostic power. In order to reduce inflation of the error rate due to the selection procedure and to limit the number of parameters in the model, a p-value of 0.01 is used as criterion for incorporation in the final prediction model. As stepwise selection methods lead to optimistic estimates of the AUC, we use cross validation to adjust the AUCs for optimism. Finally, in an attempt to simplify the final model, we also investigate the diagnostic performance of a diagnostic rule based on the two most powerful parameters of the model. Subanalysis, including only patients who completed the entire duration of the follow up and without missing data will be performed to assess robustness of the model.

Results

Baseline characteristics of the studied population

Between September 2003 and November 2006, 164 patients presenting with a hypokinetic rigid syndrome at our movement disorder clinic complied with inclusion criteria and consented to participation in this study. The demographic and baseline characteristics of the included patients are presented in Table 5.

Suspected clinical diagnosis at baseline was Parkinson's Disease in 73 patients, Multiple System Atrophy in 43, Progressive Supranuclear Palsy in 9, Dementia with Lewy bodies in 3, Vascular Parkinsonism in 30 and Corticobasal Syndrome in 6 patients.

Discussion

The large, prospective longitudinal study presented here has the potential to evaluate the diagnostic accuracy of various tests to differentiate between PD and AP in clinical practice. The diagnostic value of each of the selected ancillary investigations has been studied previously, predominantly in case-control studies. However, even the combination of these previous studies does not tell clinicians which ancillary investigation to choose when confronted with a patient with a hypokinetic-rigid syndrome of uncertain clinical etiology. This study is especially designed to evaluate the predictive value of ancillary investigations performed at baseline with the final diagnosis after follow up.

Table 5 Demographic characteristics of the included patients at baseline

Characteristics	Patients (n=164)
Age	61.3 (54.6-68.8; SD 10.2)
Males, n (%)	104 (63%)
First symptom/complaint	
- Tremor	53 (32.3%)
- Bradykinesia	19 (11.6%)
- Rigidity	16 (9.8%)
- Dystonia	3 (1.8%)
- Gait/balance disorder	23 (14%)
- Clumsiness	6 (3.7%)
- Speech	3 (1.8%)
- Pain	14 (8.5%)
- Cognitive dysfunction	1 (0.6%)
- Depression	1 (0.6%)
- Fatigue	6 (3.7%)
- Writing disturbances	7 (4.3%)
- Urogenital symptoms	4 (2.4%)
- Other	8 (4.9%)
Disease duration, months	40.6 (18.0-48.0; SD 34.3)
Disease severity	
- H&Y	2.4 (2.0-3.3; SD 0.9)
- UDPRS (III)	28.7 (17.0-37.0; SD 14.0)
- ICARS	6.9 (1.0-11.0; SD 8.0)
- MMSE	27.7 (27.0-30.0; SD 2.5)
Use of medication	
- Amantadine	11 (6.7%)
- Dopamine agonist	39 (23.8%)
- Levodopa	38 (23.2%)
- Other	2 (1.2%)
- No medication	74 (45.1%)
Care dependency[a]	64 (39%)
Use of walking aids[b]	31 (18.9%)

N: number; H&Y: Hoehn and Yahr score; MMSE: mini mental state examination; ICARS: International Cooperative Ataxia Rating Scale; UPDRS: Unified Parkinson's Disease Rating Scale; NS: not significant

[a] number of individuals that are care dependant.

[b] either walking stick, walker or wheelchair.

Data represent either number and percentage or mean, interquartile range (25 - 75%) and standard deviation.

Moreover, in addition to providing directions for the neurologist in daily practice, our study (with comprehensive assessment using validated questionnaires and clinimetric scales, both at baseline and at follow-up) will yield new insights into disease progression, succession of symptoms and may even unveil biomarkers that determine the prognosis.

Out of potential 1056 patients with a hypokinetic-rigid syndrome presenting at our movement disorder clinic, only 164 were included. This large attrition is partly explained by the function of our hospital as a tertiary referral center. Many patients are seen only once in the process of a second or even third opinion, or are referred to our clinic with a specific question regarding medication or the need for allied care by different health professionals such as physiotherapists and occupational therapists. These patients are therefore not rendered eligible for inclusion. However, although the included 164 patients only form a small and likely biased sample of the total population, our power analysis demonstrated that this sample is large enough to permit valid conclusions.

The gold standard for diagnosis of PD and the AP remains neuropathological confirmation of the clinical diagnosis upon post-mortem examination. However, prior research has demonstrated that neuropathological data show a high concurrence between the clinical diagnoses after three years follow-up and established by a movement disorder specialist with neuropathological examination post-mortem. (Hughes et al. 1992) Therefore, we use the clinical diagnosis at follow-up as the silver standard with which we compare the results of ancillary investigations in individual patients, for practical purposes. In order to minimize the potential misdiagnosis that might occur using the clinical diagnosis instead of the neuropathological diagnosis, we require the clinical diagnosis to be established in consensus by two neurologists highly experienced in movement disorders and according to the international guidelines.

4.2 A prospective study to evaluate the diagnostic approach in parkinsonism

Results of the study

Based on

MB Aerts, RAJ Esselink, WF Abdo, FJA Meijer, G Drost, N Norgren, M Janssen, GF Borm, BR Bloem, MM Verbeek, Ancillary investigations to diagnose parkinsonism; a prospective clinical study, *submitted*.

Summary

Various ancillary investigations can assist clinicians in the differential diagnosis of patients with parkinsonism. It is unknown which test offers greatest diagnostic value in clinical practice.

We included 156 consecutive patients with parkinsonism but an initially uncertain diagnosis. At baseline, all patients received the following ancillary investigations: brain magnetic resonance imaging (MRI); 123I-iodobenzamide Single Photon-Emission Computed Tomography (IBZM-SPECT); analysis of cerebrospinal fluid (CSF); and anal sphincter electromyography (EMG). The "silver standard" diagnosis (Parkinson's disease (PD) n=62 and atypical parkinsonism (AP) n=94) was established after 3-year follow-up by two movement disorder specialists, according to international consensus criteria. We determined the diagnostic value by correlating the baseline clinical parameters and ancillary studies with the silver standard diagnosis.

Out of a potential 138 parameters, univariate analysis identified 35 parameters that discriminated PD from AP, with an AUC of 0.55-0.81. Stepwise logistic regression showed that the combination of tandem gait, axial UPDRS subscore, slow saccadic eye movements and dysphagia yielded an AUC of 0.93, adjusted for optimism. The combination of tandem gait and axial UDPRS subscore yielded an AUC of 0.90. None of the ancillary investigations improved this clinically based diagnostic accuracy, not even in a subgroup of patients with the greatest diagnostic uncertainty at baseline.

Our study demonstrates that a comprehensive set of clinical tests provides good accuracy to differentiate PD from AP. Our results also suggest that routine MRI, IBZM-SPECT, CSF analysis and anal sphincter EMG do not improve this diagnostic accuracy. Future work should evaluate the possible diagnostic value of more advanced diagnostic tests.

Introduction

Due to overlap in clinical symptoms, it can be difficult to differentiate between Parkinson's disease (PD) and the various forms of atypical parkinsonism (AP), such as multiple system atrophy (MSA), progressive supranuclear palsy (PSP), dementia with Lewy bodies (DLB) and corticobasal syndrome (CBS). This differential diagnosis is especially challenging early in the course of the disease. The clinical diagnosis after several years of follow-up correlates well with the neuropathological diagnosis upon post-mortem examination. However, the diagnostic accuracy of the initial diagnosis varies greatly in PD, with accuracies of 76% in the hands of a general neurologist to up to 90% by movement disorder specialists. (Hughes et al. 1992; Hughes et al. 2002) The diagnostic accuracy is even lower for patients with a form of AP, e.g. 41-88% in PSP; (Litvan et al. 1996) and 50-66% in MSA. (Litvan et al. 1997) This uncertainty hampers optimal disease management, because a correct diagnosis is important for patient counseling and for optimizing treatment.

Various ancillary investigations are available to improve the differential diagnosis of PD and AP, including magnetic resonance imaging (MRI), (Mahlknecht et al. 2010) 123I-iodo-benzamide Single Photon-Emission Computed Tomography (IBZM-SPECT), (Vlaar et al. 2008), analysis of the cerebrospinal fluid (CSF), (Hall et al. 2012), and electromyography (EMG) (Winge et al. 2010) of the anal sphincter. Each of these ancillary investigations has been studied in selected patient populations in case-control studies. (Hall et al. 2012; Mahlknecht et al. 2010; Vlaar et al. 2008; Winge et al. 2010) However, the diagnostic value of these ancillary tests relative to clinical examination has not been studied in a design that reflects actual daily practice. Moreover, the diagnostic value of the various ancillary tests relative to each other remains unknown. Such knowledge could help clinicians to make an informed decision in selecting the best available test, knowing that many tests are time-consuming and expensive. Some tests are invasive, and not all tests are ubiquitously available.

In a prospective study involving a cohort of 156 consecutive patients with parkinsonism but an initially uncertain diagnosis, we evaluated the diagnostic accuracy and added value of detailed clinical examination and various ancillary investigations (MRI, IBZM, anal sphincter EMG and CSF analysis, all performed at baseline). Our aim was to develop a prediction model for the discrimination between PD and AP.

Methods

Patients

Between January 2003 and December 2006, all consecutive patients who had been referred to our center because of diagnostic uncertainty about the etiology of their hypokinetic-rigid syndrome were asked to participate. Our aim was to replicate everyday

clinical circumstances in general neurology clinics. For this purpose, diagnostic uncertainty was defined as being present when the community physician who referred the patient to our tertiary movement disorder centre was uncertain about the diagnosis (even when the patient in our view fulfilled the probable criteria for one of the parkinsonian syndromes). After informed consent all patients underwent a structured interview, detailed and standardized neurological examination (see **chapter 4.1** for detailed information), and, within six weeks after the initial visit, brain MRI, IBZM-SPECT, lumbar puncture and anal sphincter electromyography. After three years all patients again underwent a structured interview and the same standardized examination. Using these findings at 3-year follow-up, the final diagnosis was established by consensus agreement between by two movement disorders experts (BRB and RJE), according to current diagnostic criteria for PD, (Hughes et al. 1992) NINDS-SPSP criteria for PSP, (Litvan et al. 1996) Boeve criteria for CBS, (Boeve et al. 2003) McKeith Criteria for DLB, (McKeith et al. 2005) Gilman criteria for MSA, (Gilman et al. 2008) and ZijIlmans criteria for VaP. (ZijIlmans et al. 2004) In addition, they scored diagnostic certainty on a visual analogue scale ranging from 0% (completely uncertain) to 100% (completely certain), which enabled us to perform a planned subgroup analysis in patients with the greatest diagnostic uncertainty at baseline (diagnostic certainty of <75%). A "gold standard" diagnosis (based on post-mortem brain examination) was reached in only three patients, confirming the clinical diagnosis in all cases. We used this clinically based diagnosis after 3-year follow-up as a "silver standard" standard diagnosis. Details of the study protocol can be found in **chapter 4.1** of this thesis.
Written informed consent was obtained from the participants prior to participation of the study. All clinical investigations have been conducted according to the principles expressed in the Declaration of Helsinki. The local Institutional Review Board ("Commissie Mensgebonden Onderzoek region Arnhem-Nijmegen") approved of this consent procedure.

Ancillary investigations

Details of the MRI scan, IBZM-SPECT, lumbar puncture and anal sphincter EMG can be found in **chapter 4.1** of this thesis. Each analysis resulted in a dichotomous outcome, i.e. compliant with either PD or AP.

Statistical analysis

We analyzed the diagnostic power of all individual parameters, obtained from history and neurological assessment, both for each parameter separately and for combinations of parameters by quantification of the area under the receiver operating curve (AUC). Missing data points were imputed as normal.
Logistic regression with stepwise model selection was used to identify combinations of parameters that had optimal diagnostic power. To reduce inflation of the error rate due to the selection procedure and to limit the number of parameters in the model, a p-value of

0.01 was used as criterion for incorporation in the prediction model. As stepwise selection methods lead to optimistic estimates of the AUC, we used cross validation to adjust the AUCs for optimism. Finally, to simplify the final model, we also investigated the diagnostic performance of a diagnostic rule based on the two most powerful parameters. Subgroup analysis, including only those patients who completed the entire duration of the follow-up and who had no missing data, was performed to assess robustness of the model. Next, we used the AUC to quantify the additional value of each of the ancillary investigations, both in the entire group of patients and in a subgroup of patients with diagnostic certainty of <75%.

Results

Patient characteristics

In total 164 patients were enrolled. We excluded eight patients in whom we established a diagnosis other than neurodegenerative parkinsonism: essential tremor (n=1), dystonic tremor (n=1), neuroborreliosis (n=1), amyotrophic lateral sclerosis (n=1), psychogenic movement disorder (n=2), and pure MSA-C without signs of parkinsonism (n=2)). The final diagnoses (n=156 patients) were PD (n=62) or AP (n=94).

Forty-six patients did not complete the follow-up assessment. Reasons were as follows: too severely disabled (n=16), death (n=16), consent withdrawal (n=7), and loss to follow-up (n=7). The final diagnosis in the 110 patients who completed the 3-year follow-up were PD (n=62), MSA (n=51; 28 possible, 21 probable and 2 definite), PSP (n=12; 6 possible, 5 probable and 1 definite), CBS (n=2), DLB (n=3; 2 possible and 1 probable) and vascular parkinsonism (n=26). (Figure 1)

Baseline characteristics are shown in Table 1. Patients with a final diagnosis of AP were older and were more severely affected at baseline. Baseline characteristics showed that, compared to patients who completed the follow-up, patients lost to follow-up had a longer median disease duration and greater disease severity (reflected by the Hoehn and Yahr stages). However, the final diagnosis, using a 'last-observation-carried-forward approach' in the group of patients lost to follow-up, did not differ between both groups. (Table 2)

Out of the 110 patients who completed the 3-year follow-up, 106 patients (96.4%) underwent at least two ancillary investigations, 94 (85.7%) underwent three ancillary investigations and 58 (52.7%) underwent all four investigations.

Five patients did not receive a lumbar puncture (the main reason being use of oral anticoagulants), 20 did not receive IBZM-SPECT (the main reason being inability to withdraw medication that was incompatible with IBZM-SPECT), 20 patients did not receive an anal sphincter EMG (the main reason being previous rectal or urogenital surgery or rupture during childbirth) and 27 patients did not receive an MRI (the main reasons being

Table 1 Demographic and baseline characteristics

	Parkinson's Disease (n= 62)[xx]	Atypical Parkinsonism (n=94)[xx]	p-value
Age	56.6 (10.7)	65.0 (7.8)	<0.001
Number of men (%)	42 (67.7%)	58 (61.7%)	NS
First symptom/complaint			NS
Tremor	25 (40.3%)	25 (26.6%)	
Bradykinesia	9 (14.5%)	10 (10.6%)	
Rigidity	3 (4.8%)	13 (13.8%)	
Dystonia	2 (3.2%)	1 (1.1%)	
Gait/balance disorder	7 (11.3%)	15 (16%)	
Clumsiness	2 (3.2%)	2 (2.1%)	
Speech	0	3 (3.2%)	
Pain	7 (11.3%)	7 (7.4%)	
Fatigue	2 (3.2%)	4 (4.3%)	
Depression	0	1 (1.1%)	
Writing disturbances	3 (4.8%)	4 (4.3%)	
Urogenital symptoms	0	4 (4.3%)	
Other	2 (4.8%)	5 (5.3%)	
Distribution pattern[x]			<0.001
Asymmetrical	27 (43.5%)	8 (8.5%)	
Symmetrical	35 (56.5%)	86 (91.5%)	
Disease duration, months[x]	24 (12.7-48)	36 (18-54)	NS
Disease severity[x]			
H&Y			<0.001
1	8 (12.9%)	7 (7.4%)	
2	41 (66.1%)	15 (16.0%)	
2.5	8 (12.9%)	35 (37.2%)	
3	2 (3.2%)	23 (24.4%)	
4	1 (1.6%)	12 (12.7%)	
5	0	2 (2.1%)	
UDPRS (III)	24.1 (11.6)	32.7 (13.9)	<0.001
ICARS	2.3 (2.6)	10.2 (8.7)	<0.001
MMSE	28.4 (1.7)	27.2 (2.9)	0.002
Care dependency[x]	13 (21.0%)	31 (33.0%)	NS
Use of walking aids[x]	0	29 (30.9%)	<0.001

H&Y: Hoehn and Yahr score; MMSE: mini mental state examination; ICARS: International Cooperative Ataxia Rating Scale; UPDRS: Unified Parkinson's Disease Rating Scale

[x] At the time of inclusion

[xx] Final diagnosis after follow up

claustrophobia and metal implants; these patients received a CT-scan instead). No significant differences were observed regarding proportions of completed ancillary investigations between the PD and AP subgroups.

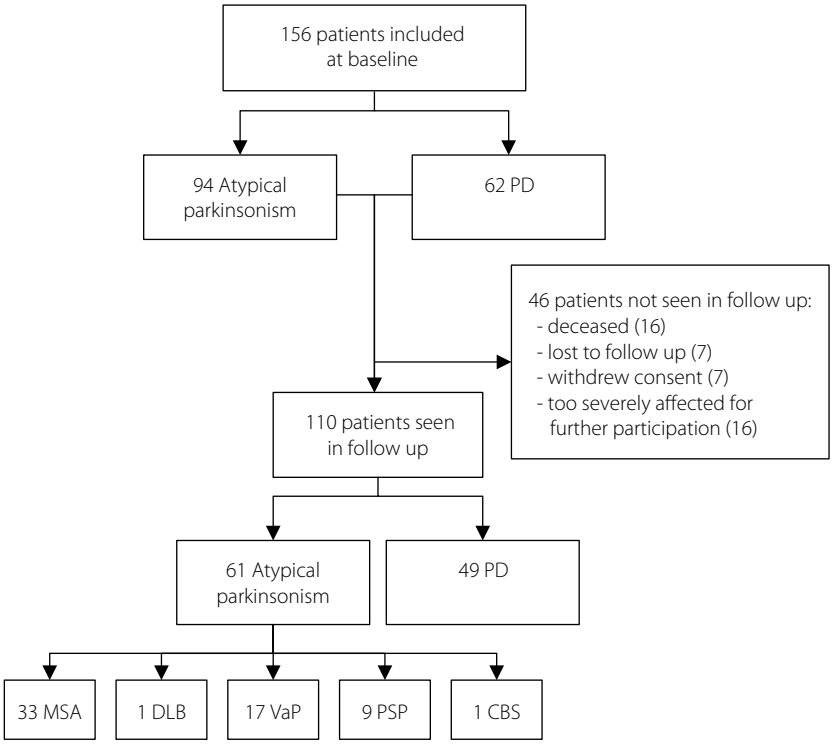

Figure 1 Flowchart of included patients.

PD: Parkinson's Disease; MSA: multiple system atrophy; DLB: dementia with Lewy bodies; VaP: vascular parkinsonism; PSP: progressive supranuclear palsy; CBS: corticobasal degeneration

The initial clinical diagnosis

In 92 out of the 110 patients who completed the 3-year follow up, the initial classification of either PD or AP at baseline was correct. Twelve patients were incorrectly diagnosed with PD at baseline, and six patients, initially classified as having AP, were diagnosed with PD upon follow-up. Moreover, the specific diagnosis (i.e., MSA, PSP, DLB, VaP or CBS) at baseline was incorrect in 33% of patients. (Table 3ab) All diagnoses (n=5) at baseline that were scored as 'probable' diagnosis remained unaltered at follow-up examination. (Figure 2)

Univariate analysis

The structured interview, neurological assessment and clinimetric scales yielded 138 clinical parameters that were potentially able to differentiate between PD and AP. Univariate analysis identified the following eight parameters that discriminated PD from AP with an AUC of at least 0.70: higher age (0.72), rapid disease progression (0.74), autonomic

Table 2 Comparison of demographic characteristics and final diagnosis between patients who completed follow up and patients lost to follow up

	Included in follow up (n=110)	Lost to follow up (n=46)	p-value
Age[x]	61.5 (10.2)	62.1 (9.4)	NS
Number of men (%)	72 (65.5%)	28 (60.9%)	NS
Diagnosis			
PD	49 (44.5%)	13 (28.3%)	NS
AP	61 (55.5%)	33 (71.7%)	
Disease duration, months[x]	28 (18-48)	40 (24-60)	<0.05
Disease severity[x]			
H&Y			<0.05
1	12 (10.9%)	3 (6.5%)	
2	45 (40.9%)	11 (23.9%)	
2.5	35 (31.8%)	8 (17.4%)	
3	12 (10.9%	13 (28.2%)	
4	5 (4.5%)	8 (17.4%)	
5	1 (0.9%)	1 (2.2%)	
UDPRS (III)	28.5 (13.3)	31.3 (3.5)	NS
ICARS	6.1 (7.2)	8.9 (9.2)	NS
MMSE	28.0 (2.3)	27.0 (2.9)	<0.05

N: number; H&Y: Hoehn and Yahr score; MMSE: mini mental state examination; ICARS: International Cooperative Ataxia Rating Scale; UPDRS: Unified Parkinson's Disease Rating Scale; PD: Parkinson's disease; AP: atypical parkinsonism; NS: not significant

[x] At the time of inclusion

Table 3a Initial clinical diagnosis compared to the final diagnosis

	Clinical diagnosis at baseline							
Final diagnosis (after follow up)		PD	MSA	PSP	DLB	VaP	CBS	Initially Misclassified patients (n)
	PD	43	3	0	0	3	0	**6 (5%)**
	MSA	6	19	1	0	6	1	**14 (13%**
	PSP	0	1	3	0	4	1	**6 (5%)**
	DLB	0	0	0	1	0	0	**0 (0%)**
	VaP	6	3	1	0	7	0	**10 (9%)**
	CBS	0	0	0	0	1	0	**1 (1%)**
	Total	55	26	5	1	21	2	**37 (33%)**

N: number; PD: Parkinson's Disease; MSA: multiple system atrophy; PSP: progressive supranuclear palsy; DLB: dementia with Lewy bodies; VaP: vascular parkinsonism; CBS: corticobasal syndrome

Table 3b Initial clinical diagnosis compared to the final diagnosis; PD vs AP

		Clinical diagnosis at baseline		
Final diagnosis		PD	AP	Misclassified patients (n)
	PD	43	6	**6 (5%)**
	AP	12	49	**12 (10%)**
	Total	55	55	**18 (15%)**

N: number; PD: Parkinson's Disease; AP: Atypical parkinsonism

dysfunction (0.75, impaired tandem gait (0.81), abnormal fluency (0.70), higher ICARS total score (0.82), (Trouillas et al. 1997) higher UPDRS axial score (0.79) and higher disease stage (0.70). (Trouillas et al. 1997) All significant univariate parameters are listed in Table 4.

Multivariate analysis

Impaired tandem gait, higher axial UPDRS subscore, presence of slowed saccadic eye movements and the presence of dysphagia were combined into a model with an AUC of 0.95 (Figure 3). After correction for optimism, the AUC was 0.93. With a cut-off value of 14.5 for this model the sensitivity was 0.88 and the specificity was 0.92. A simpler diagnostic rule with only tandem gait and the axial UPDRS subscore yielded an AUC of 0.92 (0.90 corrected for optimism) and (at a cut-off value of 13.5) a sensitivity of 0.73 with specificity of 0.92.

Subgroup analysis was performed to assess validity. Analysis of all patients, including those lost to follow-up, or including only patients who completed the follow-up assessment without any missing data, did not affect our results. Table 5 demonstrates the dependency of both positive and negative predictive values as a function of the prevalence of atypical parkinsonism.

The additional value of ancillary investigations

None of the ancillary investigations (IBZM, MRI, CSF analysis, anal sphincter EMG) increased the AUC of the clinical model. We expected the diagnostic value of ancillary investigations to be greater for patients with a higher degree of clinical diagnostic uncertainty at baseline. However, none of the ancillary investigations increased the diagnostic accuracy as compared to clinical evaluation alone in the subgroup with a baseline clinical certainty below 75% (N=61) (Table 6). Detailed data of the ancillary investigations can be found in Tables 7-9.

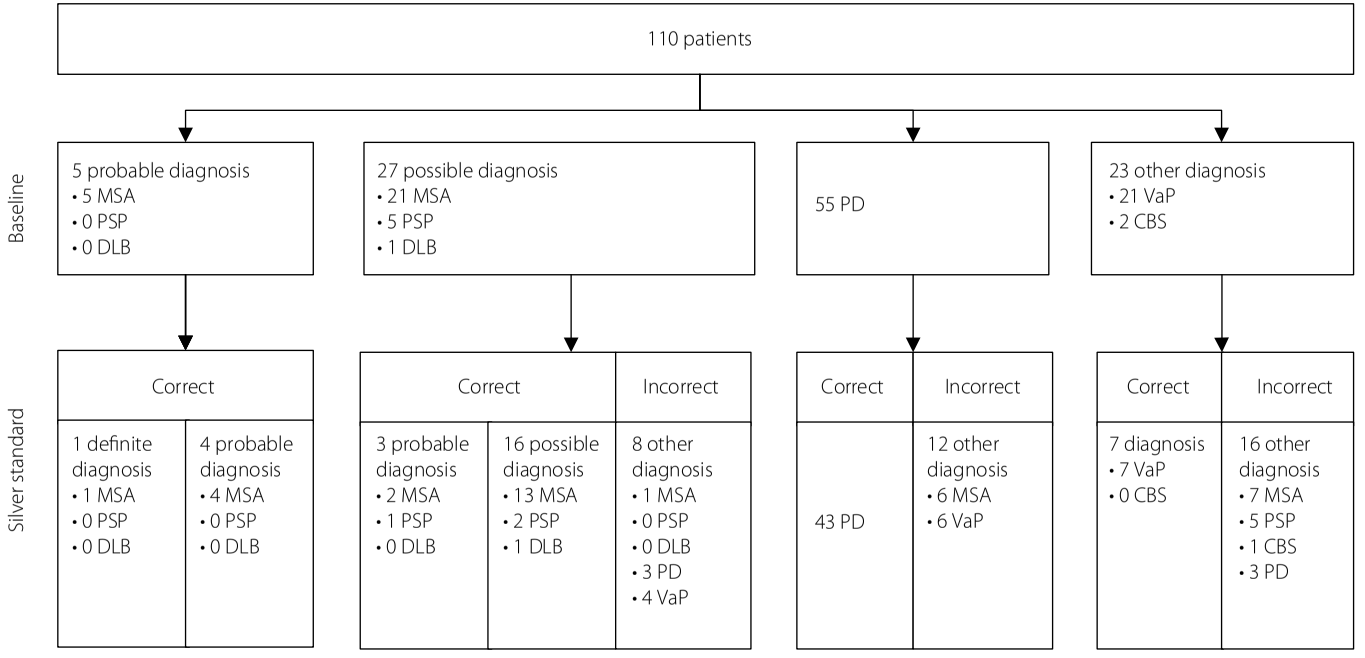

Figure 2 Clinical diagnoses at baseline and their corresponding silver standard diagnosis.

The clinical diagnoses at baseline and their corresponding silver standard diagnoses after follow up, divided in the clinical certainty at baseline (e.g. possible or probable or other) PD: Parkinson's Disease; MSA: multiple system atrophy; DLB: dementia with Lewy bodies; VaP: vascular parkinsonism; PSP: progressive supranuclear palsy; CBS: corticobasal syndrome

Table 4 Univariate analysis of parameters that discriminate between PD (n=62) and atypical parkinsonism (n=94)

Parameters	AUC	p-value
History		
Age	0.72	<0.0001
Course of the disease	0.74	<0.0001
Use of walking aids	0.65	<0.0001
Cognitive dysfunction	0.61	0.0009
Presence of ≥ 1 falls	0.68	<0.0001
Presence of night time stridor	0.55	0.0077
Hypersalivation	0.61	0.0094
Ability to cycle	0.67	<0.0001
Independence of care	0.62	<0.0001
Autonomic dysfunction	0.75	<0.0001
Dysphagia	0.64	0.0002
Neurological examination		
Romberg	0.55	0.0077
Pathological reflexes	0.63	0.0002
Cognitive assessment		
MMSE	0.61	0.0041
Fluency	0.70	<0.0001
Ataxia		
Dysarthria	0.59	0.0022
Finger-nose test	0.60	0.0005
Heel-shin test	0.58	0.0015
Tandem gait	0.81	<0.0001
ICARS total score	0.82	<0.0001
UPDRS axial score[xx]	0.79	<0.0001
Disease stage[x]	0.70	<0.0001
Schwab and England score	0.69	<0.0001
Myoclonus	0.55	0.0077
Autonomic dysfunction		
Orthostatic hypotension (directly)	0.61	0.0002
Orthostatic hypotension (after 3 min)	0.63	<0.0001
Eye movements		
Saccadic intrusions	0.68	<0.0001
Slow saccades	0.66	<0.0001
Multistep saccades	0.62	0.0004
Supranuclear palsy	0.58	0.002
Nystagmus	0.55	0.0077

AUC: area under the curve; MMSE: mini mental state examination; ICARS: International Cooperative Ataxia Rating Scale; UPDRS: Unified Parkinson's Disease Rating Scale

[x] Disease stage: 0: normal, 1: disturbed gait but walking independently; 1.5: disturbed gait, intermittent use of walking aids; 2: disturbed gait, permanent use of walking aids; 2.5: disturbed gait, intermittent use of wheelchair; 3: disturbed gait, permanent use of wheelchair; 4: death.

[xx] Axial UPDRS: a composite score of all axial parameters tested in UPDRS including: speech and face assessment, rigidity of the neck, rising from a chair, posture, gait and assessment of postural stability.

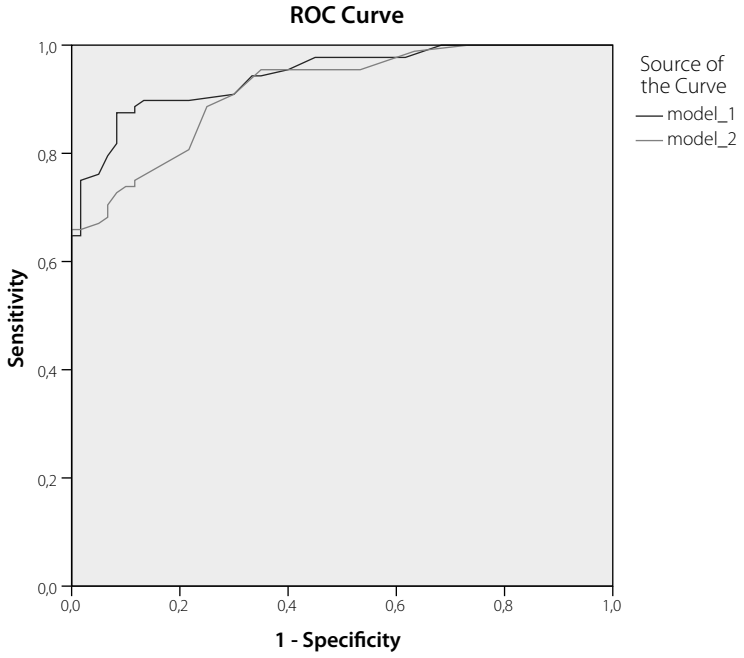

Figure 3 ROC curves for the discrimination of Parkinsons'disease and atypical Parkinsonism.

3A Receiver operating characteristic (ROC) curve analysis discriminating PD and atypical parkinsonism based on model 1: score = 11*tandem gait + UPDRS axial subscore +14* slow saccades + 5.9*dysphagia. A cut-off score of 12.5 resulted in a sensitivity of 90% and a specificity of 83%. A cut-off score of 14.5 resulted in a sensitivity of 88% and a specificity of 92%. The optimal cut-off score was calculated using the youden index.
3B ROC curve analysis discriminating PD and atypical parkinsonism based on model 2: score = 9.8*tandem gait + UPDRS axial subscore. A cut-off score of 9.5 resulted in a sensitivity of 89% and a specificity of 75%. A cut-off score of 13.5 resulted in a sensitivity of 73% and a specificity of 92%.

Table 5 Positive and negative predictive values under different assumptions of prevalence

Prevalence of atypical parkinsonism	Scenario 1 20%	Scenario 2 40%	Scenario 3* 60%	Scenario 4 80%
Model 1**				
PPV	0.73	0.88	0.94	0.98
NPV	0.97	0.92	0.83	0.66
Model 2***				
PPV	0.70	0.86	0.93	0.97
NPV	0.93	0.84	0.69	0.46

* Scenario 3 represents the sample of the present study
**Model 1: 11*tandem gait + UPDRS axial subscore +14* slow saccades + 5.9*dysphagia
***Model 2: 9.8*tandem gait + UPDRS axial subscore
PPV: positive predictive value; NPV: negative predictive value

Table 6 Diagnostic accuracy of ancillary investigations in patients with initial diagnostic certainty < 75% (N=61)

Method of investigation	AUC alone	AUC combined with the clinical model	p-value
Clinical model	0.91		
IBZM scan	0.51	0.93	NS
CSF analysis	0.61	0.92	NS
MRI scan	0.63	0.90	NS
Anal sphincter EMG	0.69	0.95	NS

AUC: area under the curve; N: number; IBZM-SPECT: 123I-iodobenzamide Single Photon-Emission Computed Tomography; CSF: cerebrospinal fluids; MRI: magnetic resonance imaging; EMG: electromyography; NS: not significant

Table 7 CSF parameters differentiating PD and AP

Parameter	PD (n=47)	AP (n= 58)	P value
Tau (ng/l)	226 (139-285)	262 (169-315)	NS (0.22)
NFL (ng/l)	1226 (726-1612)	2816(1024-3460)	<0.001
$A\beta_{42}$ (ng/l)	825 (701-942)	851 (701-1020)	NS (0.54)
MHPG (mM)	45.5 (37·0-51·0)	48.1 (38.0-54.5)	NS (0.28)
5-HIAA (mM	94.9 (70·0-115)	111 (67.0-145)	NS (0.08)
HVA (mM)	171 (99·0-205)	236 (134·0-274)	NS (0.07)

Data represent mean and 25-75% quartile range·
PD: Parkinson's disease; AP: atypical parkinsonism; n: number; NS: not significant; NFL: neurofilament light chain, $A\beta_{42}$: Amyloid β_{42}; MHPG: 3-methoxy-4-hydroxyphenylethyleneglycol; 5-HIAA: 5-hydroxyindolacetic acid; HVA: Homovanillic acid

Table 8 Sphincter ani EMG parameters differentiating PD and AP

Parameter	PD (n=40)	AP (48)	
Spontaneous activity			NS (p=0.4)*
- None	37 (92.5%)	40 (83.3%)	
- Yes	3 (7.5%)	8 (16.7%)	
Duration of the action potential			
- Normal (<10ms)	33 (82.5)	31 (64.6)	NS (p=0.1)**
- Slightly increased (10-15ms)	7 (17.5)	14 (29.2)	
- Increased /(>15ms)	0	3 (6.2)	
Amplitude of the action potential			
- Normal (<500uA)	34 (85%)	27 (56.3)	P = 0.01**
- Slightly increased (500-1000uA)	5 (12.5%)	16 (33.3)	
- Increased (>1500uA)	1 (2.5%)	5 (10.4)	
Activity pattern in contraction			
- Poor	10 (25)	24 (50%)	P= 0.03**
- Intermediate	10 (25)	12 (25)	
- Interference	20 (50%)	12 (25%)	

* as measured by Fisher's exact test
** as measured by χ^2 test
Data represent number and percentage.
PD: Parkinson's disease; AP: atypical parkinsonism; n: number; NS: not significant

Table 9 IBZM-SPECT parameters differentiating PD and AP

Parameter	PD (n= 40)	AP (n=53)	p-value
Normal	16 (40%)	21 (39.6%)	NS*
Symmetrically decreased	13 (32.5%)	19 (35.8%)	
Asymmetrically decreased	11 (27.5%)	13 (24.6%)	

Data represent number and percentage.
PD: Parkinson's disease; AP: atypical parkinsonism; n: number; NS: not significant
* as measured by χ^2 test

Discussion

We prospectively evaluated the diagnostic value of various ancillary investigations in a large cohort of PD and AP patients with an initially uncertain diagnosis. Our results suggest that the combination of tandem gait and the axial UPDRS subscore have a high predictive value to discriminate between PD and AP. Relative to this combination of clinical tests, none of the ancillary investigations (brain MRI, IBZM-SPECT, CSF analysis, anal sphincter EMG) further increased the diagnostic accuracy of PD versus the various AP syndromes. The ancillary tests were neither helpful in the subgroup of patients with greatest clinical insecurity at baseline, where one might expect most benefit from these ancillary investigations.

Out of all clinical parameters, tandem gait performance proved to be the best parameter for differentiating between PD and the group of AP syndromes (AUC 0.81). A preliminary analysis including a subset of the current cohort (36/62 PD and 49/94 AP patients) also demonstrated the diagnostic value of tandem gait assessment. (Abdo et al. 2006) However, these prior analyses were derived from a smaller population, and without confirmation of the silver standard diagnosis based on the extensive follow up that is presented here. A possible explanation for this finding is the presence of mediolateral postural instability, which is typically more pronounced in the various AP syndromes than in PD, and certainly early in the course of the disease. This mediolateral postural instability in AP can be attributed to the presence of extranigral lesions, in particular within the cerebellum, the brainstem, or their connections. (Bloem and Bhatia 2004) The diagnostic accuracy of tandem gait improved further by combination with the UPDRS axial score, i.e. the sum score of the following UPDRS part III items: face, speech, neck rigidity, rising from a chair, posture, gait, and postural instability. The UPDRS tests for gait and balance have been criticized for various reasons, (Bloem et al. 1998; Jacobs et al. 2006) but apparently still offered diagnostic value. The combination of tandem gait plus UPDRS axial score yielded a high diagnostic accuracy (AUC 0.92) that exceeded previously advocated clinical

motor and non-motor tests for the differentiation of PD and healthy controls, including the UPSIT-40 test for olfaction (AUC 0.89), the pegboard test (AUC 0.83) finger-tapping (AUC 0.75) and the Berg balance scale (AUC 0.62). (Bohnen et al. 2008) Moreover, this combination of tandem gait and axial UPDRS performed only slightly less than the diagnostic accuracy of FDG-PET (AUC for distinction between PD and AP of 0.93-0.97). (Tang et al. 2010) However, obvious disadvantages of FDG-PET are the high costs, its invasive character and limited availability.

The diagnostic accuracy of each of the ancillary investigations (brain MRI, IBZM-SPECT, CSF analysis, anal sphincter EMG) was poor and did not add to the clinical prediction model. The pathophysiological and clinical heterogeneity between the different AP syndromes may have contributed to this. For example, patients with MSA might have a hot cross bun sign on MRI, reduced tracer binding on IBZM-SPECT, elevated concentrations of CSF NFL protein and anal sphincter denervation. However, neither of these abnormalities are obligatory in other forms of AP. Hence, most investigations have a high specificity for diagnosing AP, but with a limited sensitivity. (Savoiardo 2003; Schrag et al. 2000; Yekhlef et al. 2003) Moreover, the various forms of AP may demonstrate different and sometimes even opposing abnormalities. Lumping all forms of AP therefore carries the risk of yielding a lower "net" diagnostic accuracy for each of the ancillary investigations. However, we did observe added value for brain MRI performed at baseline, in particular to improve the accuracy of the diagnosis 'vascular parkinsonism'. This demonstrates that dedicated ancillary investigations can aid in the differential diagnosis when used to answer a specific question. Current guidelines recommend using brain MRI in patients who newly present with parkinsonism, (Berardelli et al. 2013) and our results provide no reason to change this. Note that the present results were based on routine brain MRI, obtained using predominantly 1.5 Tesla scans. Future studies should evaluate the merits of more advanced MRI techniques, including high resolution scans (7 Tesla), diffusion-weighted images and diffusion tensor imaging.

Our study has several strengths. First, the study design was prospective, with a comprehensive set of clinimetric parameters and a battery of ancillary investigations (all obtained at baseline). Prior work typically examined the diagnostic merits of single ancillary tests, but we compared their relative value by applying all tests in a single cohort. Second, we included a large population of patients in whom the referring neurologist was uncertain about the diagnosis. Prior studies usually evaluated a diagnostic test in patients with a typical clinical profile and a probable diagnosis, and this may explain why the diagnostic tests performed better in those advanced populations than in our early stage patients. Third, the silver standard diagnosis in our population was established after long-term follow-up using a comprehensive, standardized assessment. We then determined which baseline test best predicted this careful follow-up diagnosis. In contrast, previous studies typically had a cross-sectional design. Finally, the sample size (n=156) was larger than most previous studies of diagnostic tests.

The gold standard diagnosis of PD and AP remains neuropathological confirmation after post-mortem brain examination. Only three patients came to post-mortem examination in this study, and the clinically based diagnosis was confirmed in all three. As an acceptable surrogate for a definite neuropathological diagnosis, we used the clinical diagnosis after 3-year follow-up as our "silver standard" reference diagnosis, as previous research has shown that the clinical diagnosis after three years of follow-up (and established by a movement disorder specialist) correlates well with neuropathological examination post-mortem. (Hughes et al. 2002) This silver standard diagnosis after three years was based upon the rate of disease progression, the treatment response, and development of any red flags, but did not include any of the baseline ancillary tests (to avoid circle reasoning). We acknowledge that a comparison of clinical parameters (obtained at baseline) to a final clinical diagnosis (established at follow-up) carries the risk of introducing circularity to the argument. Presumably, this did not affect the results much, because the clinical data at baseline and at follow-up were obtained by a single, independent examiner who was blinded to the final diagnosis (that was established in consensus by two experienced movement disorders experts who did not perform any of the clinical tests at baseline or follow-up). Moreover, the silver standard diagnosis was always established according to the international guidelines, and this does not include tandem gait as criterion. In addition, all ancillary investigations were assessed by specialists who were blinded to the clinical symptoms and the final diagnosis. Using a cut-off established at the outset of the study, these experts rated the outcome of the ancillary tests according to a dichotomous outcome, i.e. compliant with either PD or AP, to improve reliability.

Our study also had several shortcomings. First, the study cohort was not representative of the overall PD population. Out of a potential of 1056 patients with a hypokinetic-rigid syndrome who presented at our movement disorder clinic, only 164 were included. This high attrition is partly explained by the function of our hospital as a tertiary referral center. Moreover, the prevalence of patients with AP in our study was 60%, which is higher than expected based on previous epidemiological reports in the general population. (de Lau et al. 2004) The high proportion of AP patients reflects the atypical population of a neurological centre specialized in movement disorders, and this might overestimate the diagnostic accuracy of the proposed model. However, after statistical correction for background prevalence in the population, the diagnostic accuracy remains high (PPV 70%, NPV 93% at a prevalence of AP of 20%). Second, a substantial proportion (n=46; 29.5%) of patients were lost to follow-up, mainly due to death and severe disability. Based on their latest available assessment, 33 of these 46 drop-outs had a diagnosis of AP, which is understandable because of their rapid disease progression and increased mortality. Patients without complete follow-up had a shorter disease duration and greater disease severity than those with complete follow-up, and this may have affected our analyses. However, we obtained similar results when we analyzed all patients in the study using a last-observation-carried-forward approach. Finally, not all patients received all ancillary

investigations, because of contraindications such as use of anticoagulants. However, this is inevitable in a cohort of elderly subjects with age-related co-morbidity, and reflects common clinical practice.

Despite these limitations, our study suggests that when patients are referred to a specialized movement disorders center because of an uncertain diagnosis of parkinsonism, careful clinical investigations – and in particular tandem gait and the axial UPRDS subscore – suffice to distinguish AP from PD. These observations help to reduce the use of redundant, expensive and potentially harmful ancillary investigations. The high diagnostic accuracy and the simplicity of the proposed model justifies further external validation in a new cohort. In addition, further work remains needed to examine the diagnostic merits of newer and more advanced ancillary investigations, such as FDG-PET, (Tang et al. 2010) advanced MRI techniques, (Seppi and Schocke 2005) and transcranial sonography. (van de Loo et al. 2010)

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5 An illustrative example

Based on

Aerts MB, Abdo WF, Bloem BR. The "bicycle sign" for atypical parkinsonism. Lancet. 2011 8;377(9760):125-6.

Differentiation of Parkinson's disease from atypical parkinsonism is important clinically, for adequate patient counselling, and scientifically, to ascertain proper inclusion in clinical trials. The differential diagnosis remains challenging, even with current clinical insights and modern ancillary investigations. (Litvan et al. 2003) Here, we suggest that the answer to one simple question-"Can you still ride a bicycle?"-offers good diagnostic value for separating Parkinson's disease from atypical parkinsonism.

We did a prospective observational study in 156 consecutive patients with parkinsonism, but without a definitive diagnosis. At baseline, patients received a structured interview, comprehensive neurological assessment, and cerebral MRI. The interview included a standard question about whether, when, and why cycling had become impossible. The gold standard was the diagnosis after 3 years, which was based on the clinical follow-up including repeat neurological examination, response to treatment, and MRI. All assessments were done by a single, experienced examiner. All patients gave informed consent, as approved by the local ethics committee.

Before their first disease manifestation, 111 patients rode a bicycle (table 1). 45 went on to develop a gold- standard diagnosis of Parkinson's disease and 64 a form of atypical parkinsonism. At the time of inclusion (median disease duration 10 months), 34 of the 64 patients with atypical parkinsonism had stopped cycling, as opposed to only two of the 45 patients with Parkinson's disease (sensitivity 52%, specificity 96%; AUC 0.74, 95% cl 0.64-0.83). The loss of cycling abilities was present for all forms of atypical parkinsonism. Regression analysis revealed no significant effect of age, parkinsonism, or ataxia on the ability to cycle, suggesting that this was an independent marker of atypical parkinsonism. We suggest that loss of the ability to cycle after disease onset might serve as a new red flag, signalling the presence of atypical parkinsonism. The diagnostic value of the "bicycle sign" was good: its presence was highly specific for the diagnosis of atypical parkinsonism. This observation does not stand alone. Patients with Parkinson's disease have little balance problems moving sideways, (Carpenter et al. 2004) their gait is typically narrow-based, (Charlett et al. 1998) their tandem gait is usually normal, (Abdo et al. 2006) and they can show a remarkable ability to ride a bicycle. (Snijders and Bloem 2010) Cycling requires a highly coordinated interplay between balance, coordination, and rhythmic pedalling of the legs. This skilled task is probably sensitive to subtle problems with balance or coordination, caused by the more extensive extranigral pathology in atypical parkinsonism. Simply asking about cycling abilities could be added to the list of red flags that can assist clinicians in their early differential diagnosis of parkinsonism.

Table 1 Clinical characteristics

	Parkinson's disease (n=45)[a]	Atypical parkinsonism (n=66)[a]	P value
Disease subtype (%)[a]			
- Multiple system atrophy		35 (31.5%)	
- Progressive supranuclear palsy		9 (8.1%)	
- Lewy body dementia		3 (2.7%)	
- Corticobasal syndrome		2 (1.8%)	
- Vascular parkinsonism		17 (15.3%)	
Age[b]	55.5 (10.7)	65.0 (7.3)	0.001
Patients unable to cycle (%)[b]	2 (4.4 %)	34 (51.5%)	0.001
Disease duration (months)[b]	36 (20–60)	24 (12–36)	0.01
Disease severity (%)[c]			
- Stage 1	5 (11.1%)	7 (10.6%)	0.001
- Stage 2	34 (75.6%)	12 (18.2%)	
- Stage 2·5	6 (13.3%)	21 (31.8%)	
- Stage 3	0	18 (27.3%)	
- Stage 4	0	8 (12.1%)	
PIGD score (UDPRS)[b]	1.1 (0.7)	2.6 (1.3)	0.001
Posture and gait ataxia (ICARS)[b]	0.5 (0.7)	4.5 (4.1)	0.001
Oculomotor ataxia (ICARS)[b]	0.2 (0.4)	1.3 (1.2)	0.001
Limb ataxia (ICARS)[b]	1.3 (2.0)	3.8 (4.7)	0.001
Speech disorder (ICARS)[b]	0.1 (0.2)	0.7 (1.3)	0.001

Data represent means and SDs (p value assessed by use of Student's *t* test), medians and IQRs (Mann-Whitney), and number and percentage (Fisher's exact). ICARS:international cooperative ataxia rating scale, PIGD:postural instability gait difficulty. UDPRS: unified Parkinson's disease rating scale.

[a] Gold standard diagnosis, after 3 years' follow-up.

[b] At baseline, when patients were included.

[c] Modified Hoehn and Yahr stages.

6 Summary and discussion

Clinical evaluation of parkinsonism

In the first part of this thesis, we sketched the background of the clinical evaluation of Parkinson's disease (PD) and atypical parkinsonism (AP), and we provided a stepwise approach to improve the clinical differentiation.
Establishing a clinical diagnosis of *parkinsonism* can be difficult, but to differentiate between the different causes of parkinsonism can be even more challenging. The use of a systematic clinical approach, as described in **chapter 2**, may enable an adequate assessment of the patient's disorder, and may help clinicians in establishing a correct clinical diagnosis, even early in the course of the disease. This systematic approach includes three consecutive steps:

1. To verify that the clinical syndrome truly represents parkinsonism (i.e. a hypokinetic-rigid syndrome)
2. To systematically search for the presence of 'red flags' (alarm signs that may signal the presence of a form of AP)
3. To integrate these two steps, as a basis for a narrow differential diagnosis and guide for a tailored set of further ancillary tests. The importance of a careful and thorough clinical examination is reflected by the recently published EFNS/MDS-ES guideline which states that the 'diagnosis of PD is still largely based on correct identification of its clinical features'.(Berardelli et al. 2013)

Biochemistry

In the second part of this thesis we explored the diagnostic accuracy of several cerebrospinal fluid (CSF) biomarkers, including tau protein and α-synuclein for the differentiation between PD and AP. The choice of CSF parameters in these chapters was strongly influenced by the pathophysiological mechanisms in parkinsonism. The underlying pathophysiological mechanisms in PD and AP involve disturbances in the aggregation of tau protein and α-synuclein resulting in either tauopathies (corticobasal syndrome (CBS), progressive supranuclear palsy (PSP)) or α-synucleinopathies (PD, multiple system atrophy (MSA) and dementia with Lewy bodies (DLB)).

Tau protein

In **chapter 3.1** we analyzed the CSF concentrations of total protein, lactate and the brain specific proteins amyloid-β_{42} protein ($A\beta_{42}$), tau protein (t-tau), and tau protein phosphorylated at Thr181 (p-tau). These analyses were performed in CSF samples from patients with a clinically based diagnosis of PSP (n = 21), CBS(n = 12), and PD (n = 28). The

'silver' standard was the clinical diagnosis established after a mean clinical follow-up of five years (range 3 to 9 years) . A 'gold' standard diagnosis (post-mortem confirmation) was not available for these patients.
We demonstrated that the concentrations of t-tau and p-tau proteins in CSF of CBS patients were significantly elevated compared with both PSP and PD. The diagnostic accuracy of CSF t-tau and/or p-tau seems only sufficient for the discrimination of CBS vs. PD, but – as was perhaps to be expected – not for discriminating CBS vs. PSP (which are both tauopathies). However, the CSF concentrations of t-tau protein could not adequately discriminate between PD and PSP. As t-tau protein is also elevated in MSA, which is not a tauopathy, (Abdo et al. 2004) t-tau concentrations might be a biomarker for accelerated axonal degeneration in parkinsonism instead of reflecting the pathological substrate.
Some studies suggested that a decreased ratio of t-tau isoforms (33/55kD t-tau) is a good biomarker for the diagnosis of PSP (Borroni et al. 2008), especially when combined with mid-sagittal midbrain-to-pons atrophy ratio. (Borroni et al. 2010) However, attempts to reproduce these findings have not been successful (Kuiperij and Verbeek 2012), questioning the value of the proposed biomarker in clinical practice.

α-synuclein

In **chapter 3.2** we aimed to investigate whether cerebrospinal fluid (CSF) concentrations of α-synuclein have additional diagnostic value in differentiating PD from AP. One hundred and forty two consecutive patients with parkinsonism were included in this prospective study. (PD, n=58; MSA, n=47; dementia with Lewy bodies (DLB), n=3; vascular parkinsonism (VaP), n=22; PSP, n=10; CBS, n=2) Silver standard was the clinical diagnosis established after three years of clinical follow-up. CSF concentrations of α-synuclein, blood pigments and the erythrocyte count were determined.
We found similar CSF α-synuclein concentrations in patients with PD and patients with AP. This was unlikely to be a power problem given the relatively large sample size, although in our study the majority of AP were patients with MSA (an α-synucleinopathy like PD). Nevertheless, our findings suggest that α-synuclein has no value as a biomarker for the differential diagnosis of parkinsonian syndrome. However, after publication of our results, several more studies have evaluated the potential of α-synuclein for the differentiation between PD and AP. Most studies suggest a lower concentration of α-synuclein in α-synucleinopathies. (Hall et al. 2012; Hong et al. 2010; Kasuga et al. 2010; Mollenhauer et al. 2008; Mollenhauer et al. 2011; Mollenhauer et al. 2013) The comparability across these different studies describing CSF α-synuclein levels (including our own) is hindered by the use of different methods to measure CSF α-synuclein concentrations, and by the use of different antibodies in the respective assays to detect α-synuclein. Several isoforms of α-synuclein are known. However, not all isoforms are measured in the various assays that have been used. The most widely applied ELISA design/antibody combination for the quantification of total α-synuclein levels detect only full length α-synuclein and the splice

variant α-syn126 (see Figure 1). Perhaps the profile of all 4 isoforms, instead of only the full length α-synuclein and the splice variant α-syn126, would distinguish between different neurodegenerative diseases. Possibly as a result, studies have shown contradicting results. Besides different methods for detection, the observed disparity could partly be explained by differences in the selection of study populations and/ or controls, due to age effects and other possible confounders like coexisting neurological diseases. Furthermore, if any, the observed differences were small (Hong et al. 2010; Mollenhauer et al. 2008; Tokuda et al. 2006) with a profound overlap between the different patients groups, resulting in insufficient sensitivity and specificity to warrant the application of CSF α-synuclein analysis in daily practice. Recently, CSF α-synuclein was compared in de novo PD patients and healthy controls, resulting in a highly significant decrease (Mollenhauer et al. 2013) compared α-synuclein in de novo PD patients with healthy controls, describing a highly significant decrease. Nonetheless, the corresponding AUC was only 0.65 with a sensitivity of 91% and a specificity of only 25%. In another study the diagnostic accuracy was slightly higher, albeit not convincingly relevant for daily practice, with a sensitivity of 92% and a specificity of 58%. (Hong et al. 2010) Hence, it remains doubtful whether these lower α-synuclein concentrations have true value for the clinician in daily practice.

Neurotransmitter metabolites

DLB is a disease characterized not only by parkinsonism, but also by progressive dementia. In **chapter 3.3** we used CSF parameters to distinguish DLB and Alzheimer's disease (AD), as this can be equally challenging as discriminating PD and AP. We retrospectively compared CSF concentrations of the neurotransmitter metabolites homovanillic acid (HVA), 5-hydroxyindolacetic acid (5-HIAA) and 3-methoxy-4-hydroxy-phenylethyleneglycol (MHPG) and the brain-specific proteins t-tau, p-tau and $A\beta_{42}$ in 45 patients with AD (mean age 71.6 years; 34 (76%) men; 44 probable AD, 1 definite) and 23 patients with DLB (mean age 71.6 years; 18 (78%) men; 6 possible DLB, 16 probable, 1 definite). We found that CSF concentrations of 5-HIAA, HVA, MHPG, t-tau and p-tau were significantly lower in DLB than in AD, whereas CSF $A\beta_{42}$ tended to be higher in DLB. Most importantly, the combination of MHPG, p-tau, t-tau and $A\beta_{42}$ analysis discriminated between AD and DLB with high diagnostic accuracy (sensitivity 92.9%, specificity 100%). In a separate patient cohort we have confirmed that addition of MHPG to $A\beta_{42}$, t-tau, and p-tau improves the discrimination of DLB from AD, (Herbert et al. 2013) but not the differentiation of DLB from VaD or FTD. Still, CSF analysis could add to the recognition of DLB in the differential diagnosis of dementia.

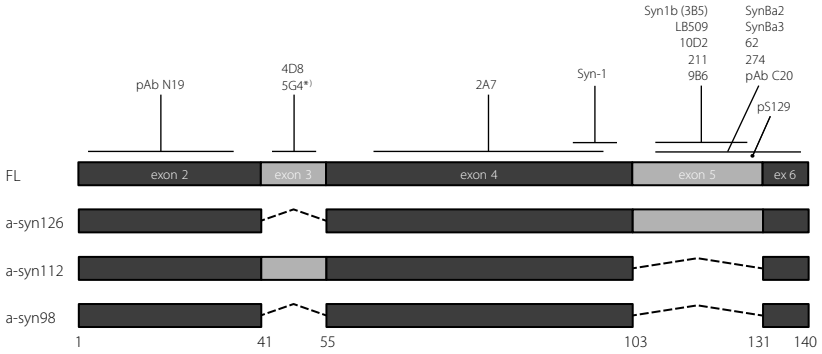

Figure 1 Illustration of the different α-synuclein isoforms. (Figure courtesy of dr. H.B. Kuiperij)

Prospective evaluation of diagnostic accuracy

In the third part of this thesis, we presented both the design and results of a prospective study to investigate the individual and relative value of clinimetrics as well as several ancillary investigations.

Study design

In **chapter 4.1** we present the study design of a prospective study to investigate the individual and relative value of electromyography (EMG) of the anal sphincter, analysis of cerebrospinal fluid (CSF), magnetic resonance imaging of the brain (MRI) and 123I-iodo-benzamide Single Photon-Emission Computed Tomography (IBZM-SPECT) for discriminating between PD and AP.

The large, prospective longitudinal study was designed with the potential to evaluate the diagnostic accuracy of various tests in differentiating between PD and AP in clinical practice. The diagnostic value of each of the selected ancillary investigations has been studied previously, predominantly in case-control studies that included patients with a clinically unambiguous presentation. However, from these previous studies it cannot be derived which type(s) of ancillary investigation are most helpful when a clinician is confronted with a patient with a hypokinetic-rigid syndrome of uncertain clinical etiology. Our trial was therefore designed with the aim to evaluate the predictive value of various ancillary investigations performed at baseline, and with the final diagnosis after follow-up. Strong elements of the design comprised the inclusion of patients with diagnostic uncertainty at baseline (in the eyes of the referring physician), the detailed and standardized

clinical work-up both at baseline and at follow-up, the use of a 'silver standard' diagnosis after three years of follow-up (using rate of disease progression, response to treatment and development of any red flags) and, in particular, the fact that multiple tests were directly compared with each other in a single design.

The results

In **chapter 4.2** we demonstrated that the combination of tandem gait and the axial UPDRS sub-score has a high predictive value to discriminate PD from AP. Out of a potential 138 parameters at baseline, univariate analysis identified eight parameters discriminating PD and AP with an area under the receiver operating curve (AUC) of at least 0.70. Stepwise logistic regression showed that the combination of tandem gait, axial subscore of the UPDRS, the presence of slowing of the saccadic eye movements and dysphasia resulted in an AUC, adjusted for optimism, of 0.93. The combination of tandem gait and the axial subscore of the UDPRS resulted in an AUC of 0.90. Interestingly, none of the ancillary investigations (CSF analysis of different biomarkers, MRI, IBZM-SPECT, and anal sphincter EMG) increased the diagnostic accuracy in differentiating PD from AP, not even in the subgroup of patients with the largest clinical insecurity of diagnosis at baseline, where one would expect the largest benefit from complementary testing.

Prior studies described a relatively high diagnostic accuracy for the various ancillary tests that are currently available (**chapters 3.1-3.3).** (Hall et al. 2012; Mahlknecht et al. 2010; Vlaar et al. 2008; Winge et al. 2010) At first sight, this appears to contrast with our observation that none of the evaluated ancillary investigations added much to the diagnostic accuracy of clinimetrics alone in differentiating between PD and AP. On the one hand, this might be explained by the high accuracy of clinimetrics in our study. Circularity can be a potential cause of this; however, prior studies (Hughes et al. 1992; Hughes et al. 2002) have demonstrated that – provided that the duration of follow-up is at least 2 years, and that the diagnosis is established by a movement disorder specialist - the correlation between the clinically based diagnosis and neuropathological findings is high (95%). As the follow-up in our study was 3 years, and since two movement disorder specialists established the final diagnosis in consensus, we have ensured at least a silver standard diagnosis to compare our data with.

Another explanation lies within the ancillary investigations itself, as the pathophysiological and clinical heterogeneity between the different AP syndromes may have contributed to this. For example, patients with MSA might have a hot cross bun sign on MRI, reduced tracer binding on IBZM-SPECT, elevated concentrations of CSF NFL protein and anal sphincter denervation. However, neither of these abnormalities are obligatory in other forms of AP. (Box 1) Hence, most investigations have a high specificity for diagnosing AP, but with a limited sensitivity. (Hall et al. 2012; Mahlknecht et al. 2010; Vlaar et al. 2008;

Box 1 Typical results of ancillary investigations in PD and atypical parkinsonism

Parkinson's Disease	
- MRI	Normal
- CSF	Decreased α-synuclein, normal t-tau, normal $A\beta_{42}$, normal NFL, normal MHPG
- EMG	Normal
- IBZM-SPECT	Increased striato-occipital ratio
Multiple system atrophy	
- MRI	Structural: putaminal rim, hot cross bun sign, decreased middle cerebellar peduncle (MCP) width, decreased pons diameter, decreased fractional anisotropy of the MCP
- CSF	Decreased α-synuclein, increased t-tau, normal $A\beta_{42}$, increased NFL, decreased MHPG
- EMG	Denervation pattern
- IBZM-SPECT	Decreased striato-occipital ratio
Dementia with Lewy bodies	
- MRI	Normal/mild atrophy of frontal, temporal and/or hippocampal region
- CSF	Normal/mildly decreased α-synuclein, normal t-tau, decreased $A\beta_{42}$, moderately increased NFL, decreased MHPG
- EMG	Mild neurogenic denervation pattern
- IBZM-SPECT	Increased striato-occipital ratio
Progressive supranuclear palsy	
- MRI	Hummingbird sign, decreased sagittal midbrain-pons ratio
- CSF	Normal α-synuclein, normal t-tau, normal $A\beta_{42}$, increased NFL
- EMG	Mild neurogenic denervation pattern
- IBZM-SPECT	Decreased striato-occipital ratio
Corticobasal syndrome	
- MRI	Asymmetric cortical frontal or frontoparietal atrophy
- CSF	Decreased α-synuclein, increased t-tau, normal/mildly decreased $A\beta_{42}$, increased NFL
- EMG	No data
- IBZM-SPECT	Decreased striato-occipital ratio

MRI: magnetic resonance imaging; CSF: cerebrospinal fluid; EMG: electromyography of the anal sphincter; IBZM-SPECT: 123I-iodobenzamide Single Photon-Emission Computed Tomography

Winge et al. 2010) Moreover, the various forms of AP, which includes a group of biochemically heterogeneous disorders (e.g. both tauopathies and α-synucleinopathies), may appear clinically similar despite the different underlying pathophysiology, and may demonstrate different, and sometimes even opposing, abnormalities in the various ancillary test. Lumping all forms of AP, therefore, carries the risk of yielding a lower "net" diagnostic accuracy than for each of the ancillary investigations, since the high specificity of ancillary tests for AP syndromes may be lost in this way.

However, we observed added value for brain MRI performed at baseline as compared to clinical / neurological investigations alone, in particular to improve the accuracy of the diagnosis 'vascular parkinsonism'. This demonstrates that dedicated ancillary investigations can aid in the differential diagnosis when used to answer a specific question. Current guidelines recommend using brain MRI in patients who newly present with parkinsonism, (Berardelli et al. 2013) and our results provide no reason to change this.
Taken together, it seems to become clear that ancillary investigations are hampered by the widely used approach to lump the different diseases together as one homogeneous form of AP. Such an approach lowers the discriminative sensitivity due to fundamental pathophysiological differences within the group of AP. One exception that we identified in this setting was the bicycle sign, which is not dependent on the specific nature of the pathophysiological disease process, but which acts as a global marker for mediolateral balance instability caused by extranigral lesions (this latter feature is a common denominator among the various forms of AP). In a research setting, this approach to lump the various forms of AP is almost inevitable. The number of patients needed to differentiate PD from any of the AP as well as all the AP independently in a prospective study would be enormous to ascertain enough patients with the individual diagnoses after follow-up.
Nonetheless, our clinical algorithm was designed to differentiate between PD and AP, but not to clarify which specific form of AP a patient has. To solve this problem, one should use the clinical examination to narrow the differential diagnosis down to two diseases, in which ancillary investigations can then be used to shift the balance. Hence, based on the prior case control studies (Hall et al. 2012; Mahlknecht et al. 2010; Winge et al. 2010) the only rational approach to employ ancillary investigations would be to employ these to answer a specific dilemma, such as differentiating PD from MSA. This approach stresses the importance of clinical assessments. In a future study, we will design and evaluate a structured clinical assessment to aid the clinician in this respect.

Illustration

The importance of clinical examination and history taking is illustrated in chapter **4.3**. Simply asking about whether or not a patient had stopped cycling yielded a high diagnostic accuracy to discriminate PD and AP. Before their first disease manifestation, 111 patients rode a bicycle. 45 went on to develop a silver-standard diagnosis of PD and 64 a form of AP. At the time of inclusion (median disease duration 10 months), 34 of the 64 patients with atypical parkinsonism had stopped cycling, as opposed to only two of the 45 patients with Parkinson's disease (sensitivity 52%, specificity 96%; AUC 0.74, 95% cl 0.64-0.83). The loss of cycling abilities was present for all forms of atypical parkinsonism.
This might be explained by the observation that balance deficits in PD are dependent on direction. Mediolateral stability is relatively preserved in patients with idiopathic PD, as

was demonstrate in dynamic posturography experiments where patients were exposed to multidirectional balance perturbations; maintaining balance sideways was hardly affected in PD patients, whereas maintaining balance in the backward was less compared to controls**.** (Carpenter et al. 2004) This preserved mediolateral stability could also explain why PD patients typically walk with a narrow-based gait and why tandem gait is preserved in most patients. (Abdo et al. 2006; Carpenter et al. 2004) In contrast, in patients presenting with a form of AP, mediolateral stability is much more affected, possibly due to more extended lesion load in AP disorders, extending beyond the nigrostriatal system.

Future perspectives

One can argue that the ancillary investigations evaluated in our study are outdated. This might be true for IBZM SPECT scanning, however, the other methods are still used in daily practice. The rationale behind these investigations was to evaluate different categories, namely structural evaluation (brain MRI), functional evaluation (IBZM-SPECT), biochemical evaluation (CSF) and clinical neurophysiological evaluation (EMG of the anal sphincter). After the design and subsequent start of the study, new techniques have become available such as diffusion tensor imaging (DTI) and functional MRI techniques,^{18}F-fluoro-deoxyglucose (FDG) positron emission tomography (PET), as well as DJ-1 analysis in CSF.

FDG PET is a novel technique, visualizing the metabolic activity in the brain. Specific disease related networks with abnormal metabolic activity have been defined for not only PD, but also MSA and PSP. In one large cohort study, FDG PET yielded high diagnostic accuracy (sensitivity >84% and specificity >91%) in the differentiation between PD and AP. (Tang et al. 2010) However, limited availability and the use of radioactive tracers remain notable disadvantages.

MRI DTI is a technique to visualize the orientation and integrity of white matter tracts, as well as gray matter areas. Another novel technique is the 7T-MRI, which enables the detailed visualization of specific brain areas such as the substantia nigra. Both techniques are not used in daily patient care yet, but have demonstrated promising results in research setting. (Kwon et al. 2012; Vaillancourt et al. 2009)

DJ-1 and the newer MRI techniques mentioned before will be subject of our future research. However, chances are that these more up-to-date ancillary investigations are hampered in exactly the same way as the investigations that were evaluated in the current study.

Conclusion

A thorough clinical evaluation remains of the utmost importance to narrow down the differential diagnosis of parkinsonism, and ancillary investigations should not be used in every patient as part of a standardized work-up. Nonetheless, this is often still the case in daily practice. In this thesis, we have established that this is not a rational approach, as the contribution over and above the clinical diagnosis is low. Moreover, most ancillary investigations are costly, invasive and some are potentially even harmful.

7 Summary in Dutch
Nederlandse samenvatting

Introductie

Dit proefschrift gaat over patiënten met de ziekte van Parkinson (ZvP) en ziekten die daar op lijken (atypische parkinsonismen, AP). De ziekte van Parkinson is een neurologische ziekte waarbij zenuwcellen in een bepaald deel van de hersenen afsterven door insluitsels van een bepaald eiwit, α-synucleïne, in die hersencellen. Deze cellen zijn een belangrijke bron van dopamine, een signaalstof in de hersenen die cellen gebruiken om met elkaar te communiceren. Patienten met ZvP zijn vaak stijf (rigiditeit), hebben moeite met het vloeiend en snel uitvoeren van bewegingen en ook met het uitvoeren van minder automatische bewegingen zoals mimiek van het gelaat (brady-/hypokinesie). Deze combinatie wordt 'hypokinetisch rigide syndroom' genoemd. Trillen (tremor), vaak van een hand of been, is een ander bekend symptoom. In de loop van de ziekte ontwikkelen patienten vaak houdingsproblemen. Deze klachten zijn (gedeeltelijk) te behandelen met tabletten die dopamine bevatten. De ziekte zelf kan vooralsnog niet geremd worden.
Er zijn ziekten die lijken op de ziekte van Parkinson maar het niet zijn (AP). Om een aantal redenen is het belangrijk om deze ziekten van ZvP te onderscheiden. Ten eerste is het verloop van deze AP veelal veel sneller en ernstiger dan bij ZvP. Ook kent ieder van de verschillende AP zijn eigen complicaties waarop je als patient en als behandelend arts bedacht zou moeten zijn. Ook voor voorlichting naar patiënten toe is dit zeer belangrijk. Ten tweede is de te verwachten respons op medicatie verschillend voor de verschillende ziektebeelden. Als laatste is het voor verder onderzoek enorm belangrijk om patiënten juist te diagnosticeren, zodat de juiste patiënten in de juiste studies geincludeerd kunnen worden.

Atypische parkinsonismen

De groep atypische parkinsonistische syndromen omvat Multipele Systeem Atrofie (MSA), Dementie met Lewy Bodies (DLB), Progressieve Supranucleaire Paralyse (PSP) en het Corticobasaal syndroom (CBS).
Bij MSA en DLB ontstaat schade, net als bij ZvP door insluitseltjes van α-synucleïne in bepaalde hersencellen, die hierdoor beschadigd raken en afsterven. Deze ziekten worden daarom samen ook wel α-synucleopathieen genoemd. Bij PSP en CBS is het niet het α-synucleïne eiwit dat problemen oplevert maar ontstaat stapeling en insluitsels van tau-eiwit op bepaalde plekken. Deze ziekten worden samen ook wel tauopathieen genoemd.
Hieronder volgt een korte beschrijving van de verschillende ziektebeelden die aan de orde komen in dit proefschrift.

Multiple systeem atrofie

Multipele Systeem Atrofie (MSA) wordt klinisch gekarakteriseerd door een combinatie van een symmetrisch hypokinetisch-rigide syndroom, cerebellaire ataxie (problemen met de coördinatie, gelijkend op dronkenschap), autonome functiestoornissen (o.a. problemen met plassen en duizeligheid bij overeindkomen) en, minder frequent, piramidebaan-

verschijnselen. De patiënten reageren over het algemeen slecht tot matig op dopamine therapie, hoewel initieel een goede respons mogelijk is. Er wordt onderscheid gemaakt tussen 2 subtypes, MSA-c wanneer cerebellaire symptomen op de voorgrond staan en MSA-p wanneer de parkinsonistische symptomen de overhand hebben. De ziekte openbaart zich meestal rond het 50ste levensjaar en is over het algemeen snel progressief. Gemiddeld worden patiënten binnen 5-6 jaar rolstoelafhankelijk. De ziekteduur is gemiddeld 8-9 jaar.

Dementie met Lewy bodies
Dementie met Lewy bodies (DLB) is een dementieel syndroom gekarakteriseerd door sterk wisselende cognitie, visuele hallucinaties, een hypokinetisch-rigide syndroom en autonome functiestoornissen. Een bijkomend symptoom is een verhoogde gevoeligheid voor bepaalde medicijnen.

Progressieve supranucleire paralyse
Progressieve Supranucleaire Paralyse (PSP) wordt klinisch gekarakteriseerd door een hypokinetisch-rigide syndroom met houdingsinstabiliteit, leidend tot frequent vallen vroeg in het ziektebeloop, cognitieve achteruitgang en problemen met het in verticale richting bewegen van de ogen. De leeftijd waarop de symptomen zich openbaren varieert van 50 tot ongeveer 70 jaar. De ziekte is snel progressief, met een gemiddelde overleving van 6-9 jaar na stellen van de diagnose.

Corticobasaal syndroom
Corticobasaal syndroom (CBS) wordt klinisch gekarakteriseerd door een asymmetrisch, slecht dopa-responsief hypokinetisch-rigide syndroom, dystonie (afwijkende stand van ledematen) en asymmetrische corticale dysfunctie (zoals problemen met de juiste volgorde van handelingen, of minder controle over de ledematen.) De ziekte komt gemiddeld zo rond het 60ste jaar tot uiting en is snel progressief met een gemiddelde overleving van 8 jaar.

De evaluatie van een patient met parkinsonisme in de klinische praktijk

In het eerste gedeelte van dit proefschrift schetsen we de achtergrond van het klinisch onderzoek bij een patient met parkinsonisme in de dagelijkse praktijk.
Het stellen van een klinische diagnose 'parkinsonisme' kan lastig zijn maar om onderscheid te maken tussen de verschillende oorzaken van parkinsonisme is vaak nog veel lastiger. Het hanteren van een systematische benadering, zoals beschreven in **hoofdstuk 2**, maakt het voor de behandelend arts gemakkelijker om belangrijke klachten en verschijnselen niet over het hoofd te zien en leidt in het algemeen tot meer nauwkeurigheid bij het stellen van een diagnose vroeger in het ziektebeloop.

Deze gestructureerde benadering bestaat uit 3 stappen. De eerste stap is, voor de hand liggend maar zeer belangrijk, om te kijken of de patient daadwerkelijk een hypokinetisch-rigide syndroom heeft. De tweede stap is dan om de patient nauwkeurig en systematisch te onderzoeken en specifiek te kijken naar 'rode vlaggen'; bepaalde symptomen die niet goed passen bij ZvP maar mogelijk duiden op een van de genoemde AP. De derde stap is het integreren van de eerste 2 stappen om zo te komen tot een einddiagnose die dan indien gewenst bevestigd of versterkt kan worden met bepaalde vormen van aanvullend onderzoek.

Biochemisch onderzoek

In het tweede deel van het proefschrift is gekeken naar de mogelijke bijdrage van het onderzoek van hersenvocht om de waarschijnlijkheidsdiagnose na het klinisch onderzoek te bevestigen. We hebben hiervoor gekeken naar diverse eiwitten in het hersenvocht van patiënten die van belang lijken te zijn bij de pathofysiologie van de verschillende ziekten.
In **hoofdstuk 3.1** hebben we gekeken naar de concentraties van onder andere tau eiwit bij patiënten met ZvP (n=28) maar ook met PSP (n=21) en CBS (n=12), beide tauopathieen. We hebben met dit onderzoek laten zien dat de concentratie van vormen van dit tau eiwit in het hersenvocht bij patiënten met CBS significant hoger is dan bij patiënten met PSP of ZvP. Het onderscheidend vermogen van deze bevinding lijkt echter alleen voldoende om patiënten met CBS te onderscheiden van patiënten met ZvP, en niet PSP en CBS patiënten onderling, of patiënten met PSP en patiënten met ZvP .
In **hoofdstuk 3.2** hebben we gekeken we naar verschillen in de concentraties van α-synucleine in het hersenvocht van 142 patienten met een hypokinetisch-rigide syndroom. Na 3 jaar follow up bleken 58 patienten ZvP te hebben, 47 patienten MSA, 3 patienten DLB, 22 patienten vasculair parkinsonisme, 10 patienten PSP en 2 patienten CBS. We hebben laten zien dat we, ondanks deze relatief grote patiëntengroep, geen verschil konden aantonen tussen de verschillende groepen patiënten. Vooralsnog heeft α-synucleine analyse van het hersenvocht dus niet voldoende onderscheidend vermogen om de verschillende ziekten van elkaar te onderscheiden.
In **hoofdstuk 3.3** hebben we gekeken naar verschillende eiwitten en neurotransmitter-metabolieten (afbraakproducten van signaalstoffen die hersencellen gebruiken om prikkels door te geven) in het hersenvocht van patiënten met DLB (n=23) en patiënten met de ziekte van Alzheimer (n=45), een andere belangrijke veroorzaker van dementie. We hebben laten zien dat de concentraties van drie belangrijke neurotransmitter-metabolieten 5-HIAA (serotonine afbraakproduct), HVA (dopamine afbraakproduct) en MHPG (noradrenaline afbraakproduct) verlaagd waren in patiënten met DLB. Door deze neurotransmittermetabolieten te combineren met de eiwitten β-amyloid en tau kon met zeer grote nauwkeurigheid onderscheid gemaakt worden tussen deze ziektebeelden.

Over het geheel genomen zijn de resultaten van de analyse van deze eiwitten, met name tau eiwit en α-synucleine in het hersenvocht teleurstellend als het gaat over het onderscheiden van ZvP versus AP. Gedeeltelijk is dit misschien te verklaren doordat in de groep AP verschillende ziektebeelden bijeen genomen worden die wat betreft pathofysiologie niet altijd even goed vergelijkbaar zijn. Kleine nuanceverschillen kunnen hierdoor verloren zijn gegaan.

De combinatie van klinisch neurologisch onderzoek en aanvullende onderzoeken

In het derde deel van het proefschrift beschrijven we de opzet **(hoofdstuk 4.1)** en resultaten **(hoofdstuk 4.2)** van een grote studie gericht op het vroeg onderscheiden van ZvP en AP. We vergeleken in deze studie de individuele waarde van verschillende vormen van aanvullend onderzoek, namelijk analyse van hersenvocht, MRI-scan van de hersenen, IBZM scan (beeldvorming van dopamine receptoren) en electromyografisch onderzoek van de sluitspier. Belangrijker dan deze individuele waarde is wellicht nog de relatieve waarde van de verschillende onderzoeken in vergelijking met elkaar en als aanvulling op het klinisch neurologisch onderzoek.
Als eerste stap werd het klinisch neurologisch onderzoek gestandaardiseerd en werden 138 verschillende parameters geïdentificeerd, variërend van de aanwezigheid van trillen van een van de ledematen tot leeftijd en geslacht. Uit deze groep van parameters bleek een aantal parameters op zich al redelijk te voorspellen of een patient ZvP of AP had. Een van de beste voorspellers bleek of een patient al dan niet 10 stapjes voetje voor voetje over een lijn kon lopen (koorddansers-gang). De combinatie van deze koorddansers-gang met een subscore van de UPDRS (het gestandaardiseerde onderzoek gericht op ZvP) bleek een AUC (area under the curve) te hebben van 0.90.
Geen van de genoemde vormen van aanvullend onderzoek bleek in staat dit verder te verhogen. Zelfs in de groep patiënten waar de neuroloog het moeilijk vond om de diagnose te stellen bleken deze vormen van aanvullend onderzoek niet bij te dragen.
De belangrijkste conclusie van deze grote studie is dan ook dat een nauwkeurig en gestandaardiseerd neurologisch onderzoek de sleutel lijkt te zijn voor een snelle en goede diagnose. Dit wordt geïllustreerd in **hoofdstuk 5** waarin wij de observatie beschrijven dat patiënten met ZvP tot lang in de ziekte nog goed kunnen fietsen, terwijl patiënten met AP daar veelal meer moeite mee hebben en daarom stoppen met fietsen. De vraag 'fietst u nog' kon bij een eerste bezoek van een patient met hypokinetisch-rigide symptomen op onze polikliniek goed voorspellen of deze patient als uiteindelijke diagnose ZvP of AP zou krijgen. Een mooi voorbeeld van het belang van anamnese en neurologisch onderzoek!

Box 1 Take home messages

- Naast de ziekte van Parkinson bestaan er ziekten die daar sterk op lijken (atypische parkinsonismen)
- Onderscheid is belangrijk voor adequate zorg, voorlichting en voor onderzoeksdoeleinden
- Een nauwkeurig neurologisch onderzoek is de kern van goede en snelle diagnose
- Aanvullend onderzoek zoals in deze studie verricht draagt hier niet of nauwelijks aan bij.

A Appendices

A.1 References

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A.2 Dankwoord

Dankwoord

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A.3 List of publications

List of publications

Herbert MK, **Aerts** MB, Kuiperij HB, Claassen JA, Spies PE, Esselink RA, Bloem BR, Verbeek MM. Addition of MHPG to Alzheimer's disease biomarkers improves differentiation of dementia with Lewy bodies from Alzheimer's disease but not other dementias. Alzheimers Dement. 2013 Nov 13. pii: S1552-5260(13)02471-0.

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Aerts MB, Synhaeve NE, Mirelman A, Bloem BR, Giladi N, Hausdorff JM. Is heart rate variability related to gait impairment in patients with Parkinson's disease? A pilot study. Parkinsonism Relat Disord. 2009 Nov;15(9):712-5.

A.4 Curriculum vitae

Curriculum vitae

Marjolein Berdina Aerts was born on January 27th 1984 in Meyrin (Switzerland). She finished her secundary education at Augustinianum, Eindhoven in 2002 (cum laude). She started medical school afterwards at the Radboud University in Nijmegen. After travelling and doing volunteer work in South Africa, she obtained her doctoral degree in 2006 (cum laude). After the regular clerkships Marjolein performed a scientific elective at the Sourasky Medical Centre, in Tel Aviv Israel in 2008 with a scholarship of the Huygens Talents Program. Here she learned Hebrew and worked with prof. Nir Giladi investigating promotor manifestations of Parkinson's disease. After graduation in 2008 (bene meritum) she worked one year as a resident in the Intensive Care department of the Elizabeth Hospital in Tilburg. In 2009 she started her PhD on improving the diagnostic aproach of PD and atypical parkinsonism in the Neurology department of the Radboud University Medical Centre under supervision of prof. Bas Bloem, dr. ing. Marcel Verbeek and dr. Rianne Esselink. In 2011 she started her training as a neurological resident, also in Nijmegen under supervision of prof. GWAM Padberg, which she hopes to finish in 2018. During the past years she has combined research and clinical training. Marjolein is married to Marvin Berrevoets. Together they have one daughter, Lauke.

Marjolein Berdina Aerts is geboren op 27 januari 1984 in Meyrin (Zwitserland). Ze volgde Voorbereidend wetenschappelijk onderwijs aan het Augustinianum in Eindhoven (cum laude). In 2002 begon zij aan haar studie Geneeskunde aan de Radboud Universiteit Nijmegen, gecombineerd met deelname aan het Honoursprogramma. Na een periode van reizen en vrijwilligerswerk in Zuid-Afrika behaalde zij haar doctoraalexamen in 2006 (cum laude). Haar co-schappen doorliep zij in Nederland, waarna zij haar wetenschappelijke stage verrichtte aan het Sourasky Medical Centre te Tel Aviv, Israel, met een beurs van het Huygens Talentenprogramma. Onder supervisie van prof. Nir Giladi deed zij onderzoek naar vroege verschijnselen van de ziekte van Parkinson. Na haar artsexamen werkte Marjolein een jaar als arts-assistent op de Intensive Care van het Elizabeth Ziekenhuis in Tilburg. In 2009 startte zij haar promotieonderzoek naar het onderscheid tussen de ziekte van parkinson en atypische parkinsonismen in de klinische praktijk bij de afdeling Neurologie van het Radboud Universitair Medisch Centrum onder begeleiding van prof Bas Bloem, dr. ing. Marcel Verbeek en dr. Rianne Esselink. Begin 2011 startte zij, ook in Nijmegen, met de opleiding tot neuroloog onder supervisie van prof. GWAM Padberg. De afgelopen jaren combineerde zij onderzoek en opleiding. Marjolein is gehuwd met Marvin Berrevoets. Samen hebben zij een dochter, Lauke.

A.5 Dissertations of the Disorders of Movement Research Group, Nijmegen

Dissertations of the Disorders of Movement Research Group, Nijmegen

Parkinson Centre Nijmegen (ParC)

1. Jasper E. Visser. The basal ganglia and postural control. Radboud University Nijmegen, 17 June 2008
2. Maaike Bakker. Supraspinal control of walking: lessons from motor imagery. Radboud University Nijmegen, 27 May 2009
3. W. Farid Abdo. Parkinsonism: possible solutions to a diagnostic challenge. Radboud University Nijmegen, 7 October 2009
4. Samyra H.J. Keus. Physiotherapy in Parkinson's disease: towards evidence-based practice. Leiden University, 29 April 2010
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